
Culture of striped trumpeter
(*Latris lineata*) post-larvae

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Doctor of Philosophy

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I declare that the content and the manuscripts published from this thesis are the products of my own work. I was responsible for executing experiments, sampling, data collection, laboratory analysis, analysing the data, writing draft manuscripts and thesis chapters, submission to peer review journals and incorporating revisions into the final written product.

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Dr Jennifer Cobcroft (Marine Research Laboratories, Taroona) measured larvae and assessed the severity of jaw malformations of the sampled animals. Associate Professor Stephen Battaglione, Ross Goldsmid and Anna Overweter provided assistance with live feed production and during sampling. Dr Matthew Bransden (Skretting – Australia) provided the microdiets used for the experiment.

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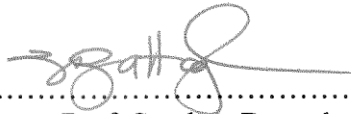
Ross Goldsmid, Alan Beech, Anna Overweter, Bill Wilkinson and Debbie Gardner assisted me during sampling days. Dr Thomas Rodemann (University of Tasmania) performed elemental analysis. Dr Robin Katersky (University of Tasmania) coordinated the manufacturing and analysis of diets used for the experiment.


Chapter 5: Modelling nutrient requirements of post-larval striped trumpeter (*Latris lineata*)


Dr Thomas Rodemann (University of Tasmania) performed elemental analysis on the samples.

Chapter 6: Chemical composition of striped trumpeter (*Latris lineata*) throughout its life-cycle

Dr Ashley Townsend (University of Tasmania) performed the elemental analysis via ICP-OES. Daniel Pountney (University of Tasmania) performed the acid digestion. Dr Thomas Rodemann (University of Tasmania) performed elemental analysis on the samples.

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Abstract

The striped trumpeter (*Latris lineata*) has been the subject of research at the Marine Research Laboratories, Taroona since the 1980s as an alternative species for the Tasmanian Atlantic salmon (*Salmo salar*) farming industry. It is an endemic species with excellent white flesh, high in polyunsaturated fatty acids. Considerable research has been conducted into improving larval survival and quality through better understanding of optimal rearing conditions, nutrition and health. The culture of juvenile striped trumpeter is complicated by a prolonged post-larval or “paperfish” stage which can last up to nine months. My study is the first to research the nutrition and optimal rearing conditions of striped trumpeter post-larvae. Prior to this study, post-larvae were weaned from live feeds at three months of age. Research showed post-larvae could be weaned onto formulated diets using a co-feeding strategy at 40 days post-hatch (dph) and could be fully weaned onto microdiets at 50 dph. Feeding larvae with live feeds led to higher survival and growth but the use of live feeds required more financial and human resources and did not have a consistent nutritional profile. The adoption of feeding formulated diets to larvae by 50 dph has greatly increased production of post-larvae. Temperature regime was found to be the most significant factor influencing growth and development of post-larvae. Three-hundred-day-old post hatch post-larvae (12.1 ± 0.2 g, mean \pm SE) were reared at 12, 14, 16 and 18 °C, over 84 days. Polynomial models predicted that growth was maximised between 12.9 °C (thermal growth coefficient) and 14.4 °C (specific growth rate). Post-larvae reared at 16 °C exhibited similar growth rates but did not metamorphose into juveniles as quickly as post-larvae reared at 14 °C and 12 °C. Post-larvae reared at 18 °C showed the slowest growth and metamorphosis rates.

Post-larvae reared at temperatures outside the optimum required more nutrients to cope with the increased metabolic demand and this was reflected in their whole body chemical composition and productive protein and energy values. The effects of ration were studied by feeding two-hundred-eighty-seven day-old post-larvae (8.1 ± 0.1 g, mean \pm SE) at three ration levels, 33 %, 67 % and 100 % satiation, using dietary lipid levels of 18 % and 24 %, over a period of 63 days at 15 °C. Polynomial models predicted a feeding rate of 4 % biomass day⁻¹ to be optimal. Growth, total carcass crude lipid and development into juveniles were influenced by ration level. Dietary lipid levels did not affect growth and development but had a significant effect on carcass total lipid. Minimum thresholds for metamorphosis into juveniles were determined to be 20 g in wet weight and at least 4 % carcass total lipid. Metabolic weight exponents for protein and energy were derived from data collected using starvation trials of ten days in duration on fish of 1 g to 120 g wet weight. Metabolic weight exponents of 0.8 ± 0.2 for energy and 0.6 ± 0.0 for protein were found. A factorial model was used to predict protein and energy requirements of post-larvae via data from the temperature and ration growth trials. Maintenance requirements were 1.8 g body weight^{-0.7} day⁻¹ of protein and 116.1 kJ body weight^{-0.8} day⁻¹ of energy. An optimum dietary protein to dietary energy ratio of 21.8 g protein MJ⁻¹ was predicted. My study provides the first empirical data to develop protocols for the rearing of striped trumpeter post-larvae. The results have been successfully used to produce over 5,000 juveniles grown out in pilot sea cage trials in Tasmania. Whole body chemical, mineral and trace elemental composition data from striped trumpeter ranging from 1 g to sexually mature broodstock were collected. The accretion of whole body total lipid was inversely related to moisture while protein and ash content remained constant. Models were developed which predict the whole

body chemical, mineral and trace elemental composition of striped trumpeter. My study has important implications for aquaculture and fisheries management of striped trumpeter, in particular the rearing of post-larvae in hatcheries, timing of stocking into sea cages, especially prior to metamorphosis, and for wild stock recruitment models.

Chapter 1

General Introduction

1.1 *World aquaculture*

Aquaculture is the fastest growing primary industry worldwide and will continue to grow steadily with an increasing global demand for seafood (De Silva, 2001; Carter, 2007). Production of seafood from capture fisheries has not increased in over ten years from 94.6 million tonnes in 1996 (FAO, 1999) to 92.0 million tonnes in 2006 (FAO, 2008). Wild fisheries are heavily exploited and in some cases exploited beyond capacity (Myers and Worm, 2003; Clover, 2005). The outlook for wild capture fisheries production is bleak and is predicted by some to collapse by 2048 (Worm et al., 2006). In contrast, aquaculture production has grown by 6.9 % annually since 1970. Aquaculture's share of global seafood production increased from 20 % (26.4 million tonnes) in 1996 to 47 % (51.7 million tonnes) in 2006 and is projected to reach 50 % in the near future (FAO 1999, 2008). Aquaculture is not only an important industry in terms of food security but also because of its economic value. The latest statistics from the Food and Agriculture Organization (2008) show that in 2006, aquaculture production was valued at US\$ 78.8 billion (AU\$ 87.0 billion). This is further divided into freshwater aquaculture production which accounts for the largest share of total aquaculture at 58 % (15.1 million tonnes). Production in marine environments accounted for 34 % by volume (9.7 million tonnes) and 36 % by value while brackish water production accounted for 8 % by volume (1.6 million tonnes) and 16 % by value. In terms of value, freshwater aquaculture accounts for 48 % (US\$ 37.8 billion); marine aquaculture accounts for 36 % (US\$ 28.4 billion) and brackish aquaculture accounted for 16 % (US\$ 12.6 billion).

1.2 Aquaculture in Australia and Tasmania

The Australian aquaculture industry contributes significantly to the growth of the country's economy. In 1998, the value of Australian aquaculture production was AU\$ 613.6 million (O'Sullivan and Dobson, 2000). This figure has grown, albeit more slowly than global growth, to AU\$ 868 million in 2008 (ABARE, 2008). Aquaculture of finfish is the most important sector and accounts for the largest share of production and volume. The two most important finfish species are Southern bluefin tuna (*Thunnus maccoyii*) and Atlantic salmon (*Salmo salar*). The Southern bluefin tuna industry is concentrated in South Australia and the Atlantic salmon industry in Tasmania. In 2008, production of Southern bluefin tuna was 14,700 tonnes valued at AU\$ 210 million while production of Atlantic salmon was 26,000 tonnes (ABARE, 2008) valued at AU\$ 272 million (Battaglene et al., 2008).

Tasmania is Australia's smallest state but accounts for the largest share of Australian seafood production (22 %) (ABARE, 2008). The value of Tasmania's seafood production was AU\$ 475.5 million in 2007, of this aquaculture production accounted for AU\$ 319 million or a 67 % share. Three species contribute to aquaculture production, abalone, oysters and Atlantic salmon and of the three, Atlantic salmon aquaculture is the dominant industry. The Atlantic salmon industry has continued to grow from strength to strength, from producing 7,072 tonnes in 1998 to 26,000 tonnes in 2008, partly due to increased marketing efforts and strengthening domestic consumption but also due to the research effort to improve feeding efficiency and husbandry. Growth prospects of the industry are strong and industry production is aiming to grow by 12.5 % annually until 2013 (Battaglene et al., 2008).

The success of marine finfish aquaculture has led to the development of new species for aquaculture in many states driven by several factors including national regional strategic development, a need for regional employment and development, and individual entrepreneurship (Battaglione and Kolkovski, 2005) (Table 1.1). The importance of marine finfish aquaculture for Australia is predicted to grow as more species and markets are developed (Kolkovski et al., 2004; Battaglione and Kolkovski, 2005).

Table 1.1: Established and new marine finfish species produced or being investigated in Australia (modified from Battaglione et al., 2008).

Common name	Scientific name	Climate	State
Atlantic salmon	<i>Salmo salar</i>	Coldwater	TAS
Ocean trout	<i>Oncorhynchus mykiss</i>	Coldwater	TAS
Brook trout	<i>Salvelinus fontinalis</i>	Coldwater	TAS
Greenback flounder	<i>Rhombosolea tapirina</i>	Coldwater	TAS
Long-snout flounder	<i>Ammotretis rostratus</i>	Coldwater	TAS
Striped trumpeter	<i>Latris lineata</i>	Coldwater	TAS
Banded morwong	<i>Cheilodactylus spectabilis</i>	Coldwater	TAS
Yellowtail kingfish	<i>Seriola lalandi</i>	Temp/Cold	SA,WA,NSW
Black bream	<i>Acanthopagrus butcheri</i>	Temp/Cold	TAS,WA
Yellowfin bream	<i>Acanthopagrus australis</i>	Temperate	QLD
Southern bluefin tuna	<i>Thunnus maccoyii</i>	Temperate	SA
Australian bass	<i>Macquaria novemaculeata</i>	Temperate	NSW,QLD
Snapper	<i>Pagrus auratus</i>	Temperate	NSW, SA,WA
Mullocky	<i>Argyrosomus japonicus</i>	Temperate	NSW,SA
Sand whiting	<i>Sillago ciliata</i>	Temperate	NSW,QLD
Trumpeter whiting	<i>Sillago maculeata</i>	Temperate	NSW,QLD
West Australian dhufish	<i>Glaucosoma hebraicum</i>	Temperate	WA
Dolphin fish	<i>Coryphaena hippurus</i>	Trop/Temp	WA
Pikey bream	<i>Acanthopagrus berda</i>	Tropical	QLD
Barramundi	<i>Lates calcarifer</i>	Tropical	QLD,SA,NSW
Coral trout	<i>Plectropomus spp.</i>	Tropical	QLD
Golden snapper	<i>Lutjanus johnii</i>	Tropical	NT,QLD
Mangrove jack	<i>Lutjanus argentimaculatus</i>	Tropical	QLD
Grouper	<i>Epinephelus spp.</i>	Tropical	QLD,WA

1.3 Diversification, is it necessary?

There is an old adage that says “If it isn’t broken don’t fix it.” However, diversification is a prudent economic strategy to minimise risk and uncertainty. Wilson and Archer (2009) discussed the risk of widespread monoculture and a standardisation of techniques and species which exposes the industry to possible collapse as the pathogen and parasite biomass grows along with the cultured species and increases opportunities for adaptation and attack on the host species. The Chilean salmon industry is a recent example where an ISA (infectious salmon anaemia) outbreak and sea lice infestation decimated the salmon population leading to loss of employment, closure of several salmon farming companies and the loss of confidence in the Chilean product (Bravo, 2003; Barrionuevo, 2009). The development of a new species also makes economic sense by tapping new markets instead of competing in a crowded market space (Le Francois et al., 2009). Dallimore (2005) presents an example where the selling price of European seabass (*Dicentrarchus labrax*) dropped from € 12 kg⁻¹ to € 4 kg⁻¹ when production increased from 20,000 tonnes to 200,000 tonnes. Diversification is not imperative but in the face of multiple risks the benefits of diversification are great.

Development of new species for aquaculture should select those species which can thrive in the local climate and environment and should also show robust commercial potential unaffected by the vagaries of the economic climate supported by a scientific understanding of the requirements for its successful culture (Le Francois et al., 2009). The rapid expansion and development of aquaculture need to be tempered with an awareness of its potential effects on the aquatic environment and its impact on the utilisation of resources. Aquaculture has been criticised for its

environmental impacts on the seabed resulting from fish waste and excess feed, its reliance on fish meal and fish oil, and as a potential health hazard for wild populations of fish species (Naylor et al., 2000; Bjorn et al., 2001, Miller et al., 2008). Aquaculture development should therefore adopt a considered and researched approach to avoid the potential pitfalls. The lack of scientific biological information and the competition from wild catch has caused several of the largest companies involved in Atlantic cod (*Gadus morhua*) farming to abandon their efforts, this was caused by a combination of malformations in hatchery produced fish and slow growth rates leading to delayed revenues which were further challenged by a resurgent supply of wild caught cod (Solsletten et al., 2009).

The Tasmanian aquaculture industry plans to leverage its research capabilities and pristine marine environment to develop a high-value product that will offer a competitive advantage over mass cultured low value seafood products (Battaglione et al., 2008).

1.4 *The striped trumpeter: a new species for aquaculture*

In a review by Searle and Zacharin (1994) the striped trumpeter (*Latris lineata*) was selected as the best candidate for development for sea cage culture in Tasmania. It is a highly regarded food fish and can be prepared in a variety of ways including *sashimi* (Yearsley et al., 1999). The striped trumpeter is endemic to Tasmanian waters with a reported distribution around the temperate regions of the southern Atlantic, Pacific and Indian oceans to encompass the coastal regions of New Zealand, the Gough and Tristan Da Cunha Island groups (Atlantic Ocean), the Amsterdam and St. Paul Island groups (Indian Ocean), and the Foundation seamount

(Pacific Ocean) (Last et al., 1983; Duhamel, 1989; Kingsford et al., 1989; Andrew et al., 1995; Roberts, 2003) (Fig. 1.1).

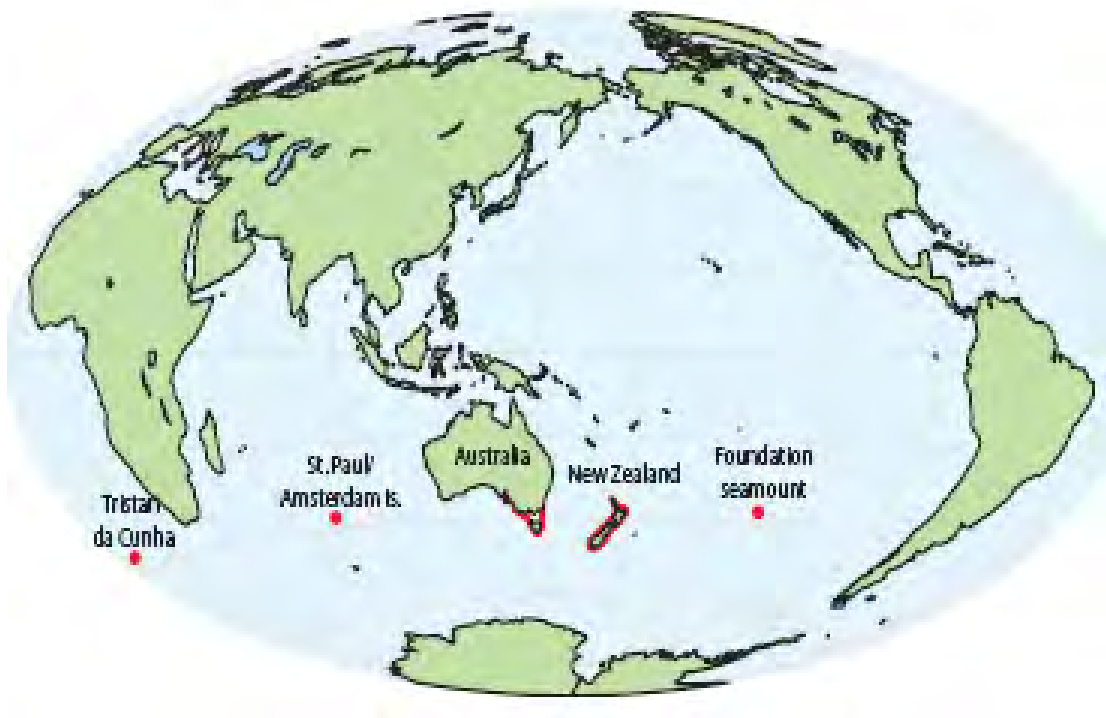


Figure 1.1: The known range of worldwide distribution of the striped trumpeter (indicated in red shading) (adapted from Tracey, 2007).

The striped trumpeter is found on the continental shelf over rocky bottoms to depths of 300 m with the juveniles associated with shallow inshore reefs. The juveniles remain in the shallow reefs and do not move into deeper offshore reefs until they are 45 cm in size. Striped trumpeter can grow up to 1.2 m in length and 25 kg in weight. Sexual maturity is reached at 6.8 years for females and 6.2 years for males. (Ziegler and Lyle, 2009).

The current market price (November, 2009) of striped trumpeter fillets is close to AU\$ 40.00 kg⁻¹. Fillets contain one of the highest concentrations of omega-3 among commercially available fish species which should further increase its appeal

(Nichols et al., 2005). The consumption of long chain omega-3 polyunsaturated fatty acids are associated with many benefits for human cardiovascular and developmental health (Cohen et al., 2005; Hibbeln et al., 2007; Golding et al., 2009). Wild fisheries production of striped trumpeter does not supply significant commercial quantities of striped trumpeter; supply peaked at 110 tonnes in the early 1990s but decreased to 70 to 80 tonnes in the mid 1990s, and is now less than 20 tonnes (Table 1.2) (Lyle, 2005; Ziegler et al., 2006; Tracey, 2007, Ziegler and Lyle, 2009). The reasons for the decline are not fully understood but include overfishing and poor juvenile recruitment.

Table 1.2. Annual commercial catches of striped trumpeter (tonnes) south of latitude 39° 12"S. (Ziegler and Lyle, 2009)

Year	Tasmania	Victoria	Commonwealth	Total
1990 to 1991	74.5	37.1		111.6
1991 to 1992	58.2	36.8		95.0
1992 to 1993	52.7	19.8		72.5
1993 to 1994	56.5	16.0		72.5
1994 to 1995	72.4	14.6		87.0
1995 to 1996	60.3			60.3
1996 to 1997	79.7		0.7	80.4
1997 to 1998	75.4		5.7	81.1
1998 to 1999	98.4		8.9	107.4
1999 to 2000	86.3		14.5	101.8
2000 to 2001	41.2		7.5	49.6
2001 to 2002	40.0		4.8	44.8
2002 to 2003	36.8		3.2	40.0
2003 to 2004	36.8		3.7	40.5
2004 to 2005	24.0		2.2	26.2
2005 to 2006	19.1		4.7	23.8
2006 to 2007	18.7		3.5	22.2
2007 to 2008	12.5		3.0	15.5

Given the limited capacity of capture fisheries production and the high demand for striped trumpeter, aquaculture is arguably the only means of meeting a high demand for this species. Continuous research on striped trumpeter has improved

the hatchery and husbandry techniques and closed the life cycle (Fig. 1.2). The optimum temperature for egg incubation has been identified and combined with the use of ozone as a disinfectant have resulted in high hatching rates (Bermudes and Ritar, 1999, Morehead and Hart, 2003, Battaglione and Morehead 2006). Studies have also been undertaken into larval sensory organ development which improved swim bladder inflation, previously a cause of mass mortalities (Trotter et al., 2001; Cobcroft and Pankhurst, 2003). Larval feeding was improved by investigating the effects of turbidity (Shaw, 2006) Research has also identified important pathogens, such as *Kudoa neurophila* (Grossel, 2005) and copepodid parasites which may infect animals during culture (Tang et al., 2007; Andrews, 2010). Recent research has investigated the development of the immune system with a view towards vaccination development (Covello et al., 2009). The combined research effort has culminated in the completion of the first sea cage trials in 2009.

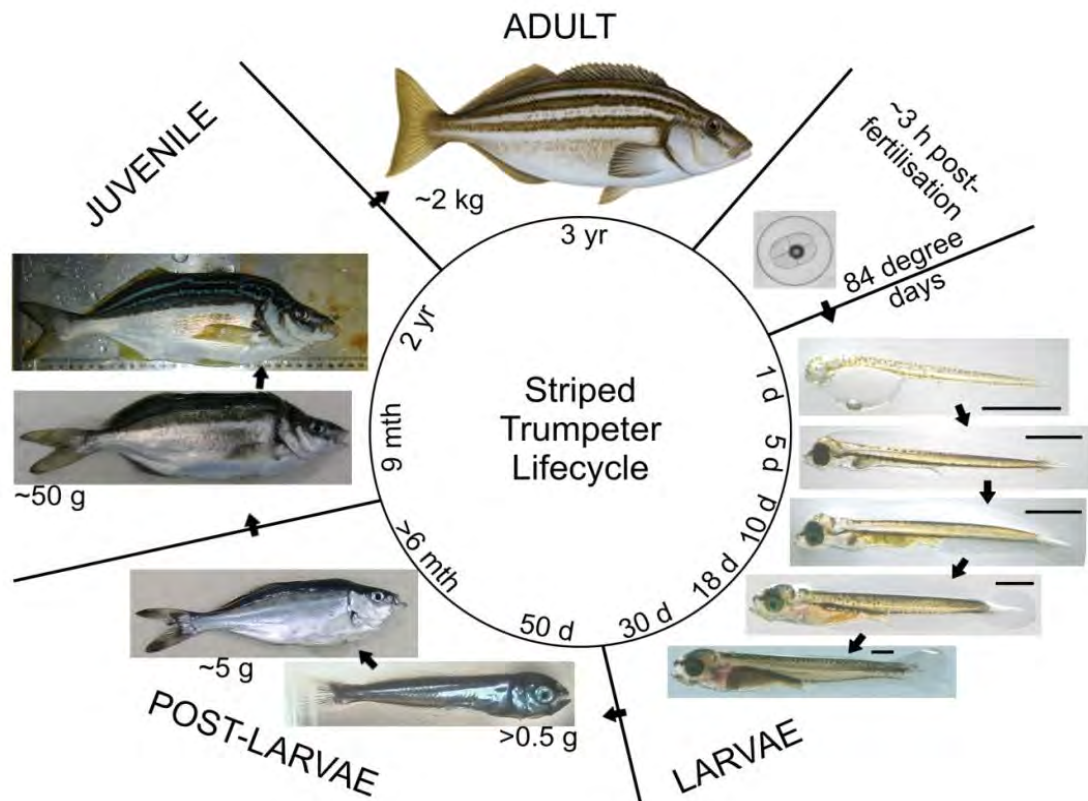


Figure 1.2: Life history of the striped trumpeter in captivity with estimated age and weights, scale bars are 1 mm (Andrews, 2010).

Considerable challenges still remain, one of these relates to a complex life-history. The striped trumpeter undergoes a prolonged post-larval or “paperfish” stage which can last up to nine months (Furlani and Ruwald, 1999; Tracey and Lyle, 2005). This pattern of development is unusual but is also observed in the banded morwong (*Cheilodactylus spectabilis*), a closely related species (Ritar and Pribadi, 2006). Cultured post-larvae begin to metamorphose into the juvenile form at 120 mm total length and into the adult form, with its distinctive striped pattern, at 180 to 200 mm (Tracey, 2007). Jaw malformations first identified in the larvae but which persist until adulthood remain an issue (Cobcroft et al., 2001). The “walling” activity of

larvae and post-larvae is suspected to be one of the causes of jaw malformations and has recently been investigated and appropriate tank colours and patterns have been identified (Cobcroft and Battaglione, 2009). The post-larvae are more susceptible to stress induced mortality and precautions must be taken to prevent mass mortalities (Battaglione and Cobcroft, 2007). Research on post-larvae has only been possible recently following the reliable production of larvae (Battaglione and Cobcroft, 2007). Production has enabled the research into the protein, lipid and energy requirements of post-larvae and the possibility of conducting relatively large scale replicated experiments to understand post-larval growth and husbandry and how these can be improved in culture.

1.5 *Striped trumpeter nutrition research*

The production of high quality juveniles is a prerequisite for the successful implementation of an aquaculture enterprise (Battaglione and Fielder, 1997) and nutrition is an important component of successful juvenile production. Nutrition techniques have been applied to gauge the effectiveness of striped trumpeter broodstock rearing techniques by comparing the quality of eggs produced by captive broodstock with those from wild populations (Morehead et al., 2001). Morehead et al., (2001) found little difference in total lipid between eggs from captive and wild broodstock sources although the polyunsaturated fatty acid profile was related to the diet. A broodstock diet manufactured on-site was adopted as a standard broodstock diet in 2004 (Battaglione and Cobcroft, 2007). A study by Bransden et al., (2007) on broodstock condition and egg quality concluded that captive broodstock produce eggs of excellent quality.

The majority of nutrition research has necessarily concerned larviculture. Providing adequate nutrition from first feeding to metamorphosis is crucial because larvae and post-larvae encounter energy crises during developmental intervals (Thorisson, 1994). Practical improvements in live feed enrichment techniques and products increased survival of striped trumpeter larvae (Bransden et al., 2004a,b Battaglione and Cobcroft, 2007). This led to large scale replicated trials on live feed enrichment to manipulate fatty acid profiles. Bransden et al., (2005a,b) identified the optimum level of DHA in live feeds to support larval development. During the rotifer feeding stage, feeding below the optimum level of 12.7 mg DHA g⁻¹ dry matter resulted in the impairment of visual ability and abnormal changes in the gut and liver (Bransden et al., 2005a). During the *Artemia* feeding stage, the DHA requirement increased to 20.8 mg DHA g⁻¹ dry matter. Studies on changes in chemical composition of striped trumpeter larvae were conducted by Brown et al., (2005a,b) that identified changes in whole animal amino acid and vitamin concentrations (ascorbic acid and alpha-tocopherol) during early larval growth. Prior to my study there has been no research on striped trumpeter post-larvae.

1.6 Scope and objectives

The success of research on striped trumpeter larval nutrition has established techniques for larviculture but improvements in growth and survival are continuously sought. The reliance on live feeds entails investment in personnel and rearing facilities which increases the cost of production (Le Ruyet et al., 1993). Live feed are unlikely to meet nutrient requirements in a balanced way and Bransden et al., (2005b) also found that the fatty acid profile of *Artemia* is not ideal for striped

trumpeter larvae. The development of formulated diets for larvae using more sophisticated manufacturing techniques has led to a decrease on the reliance of live feeds (Curnow et al., 2006) and for some marine finfish species the complete elimination of the use of live feeds during larviculture (Cahu et al., 2003). The use of formulated diets for larviculture is also beneficial from a cost and efficiency perspective because it obviates the need for additional human resources and facilities, is simple to use and store, and maintains a consistent nutritional profile (Cahu and Zambonino Infante, 2001). Weaning is the process wherein larvae are transitioned onto feeding on inert formulated diets. A gradual transition using a co-feeding strategy, slowly replacing live feeds with formulated diets (Rosenlund et al., 1997) or an abrupt and complete shift onto formulated diets are both traditionally used. Prior to the current study, weaning of striped trumpeter post-larvae was completed at 100 days post-hatch (dph) (Battaglione and Cobcroft, 2007). Recent improvements in feeding systems (Curnow et al., 2006) and new generation commercial feeds (e.g. Gemma Micro™, Skretting) have not been tested on striped trumpeter post-larvae prior to the current study.

The synthesis of all of the previous research studies and practical improvements in culture methodology has resulted in the consistent production of striped trumpeter post-larvae and juveniles (Battaglione and Cobcroft, 2007). Striped trumpeter post-larvae are delicate animals and because of the prolonged duration of this life-history stage it is important for the continued progress of the research program to shorten its duration and to decrease mortalities. Temperature is widely accepted as having a profound influence on the growth rates of fishes (Brett, 1979; Jobling, 1994). Identifying the optimum temperature for rearing is important for its impact on growth rates and the prevention of detrimental effects, such as

malformations which can develop at temperatures beyond the optimum (Wang and Tsai, 2000; Sfakianakis et al., 2006; Katersky and Carter, 2007). Previous studies have identified 12 °C to 14 °C as the optimum temperatures for egg incubation (Bermudes and Ritar, 1999; Morehead and Hart, 2003) and 16 °C for the rearing of larvae (Trotter et al., 2003), however, no research had been conducted on the optimum temperature for rearing post-larvae.

Clearly, developing suitable feeds and feeding are critical to avoid a condition of sub-optimum nutrition (Brett and Groves, 1979; Bureau et al., 2006). Inadequate feed results in slow or even negligible growth, delayed development and can increase aggression among animals (Carter et al., 1992; Berrill et al., 2006). The provision of too much food leads to increased organic loading and the production of harmful compounds (i.e. hydrogen sulphide) and also wastage which increases production costs and has negative impacts on the environment and potentially on fish health (Wu et al., 1994; Black et al., 1996; Lovell, 2002). Feed management and proper nutrition ensures a healthy immune response in fish (Lim et al., 2005).

Factorial modelling is an approach to express requirements in relation to intake and retention and develops allometric relationships using metabolic weight exponents for nutrients of interest (Hauler and Carter, 2001). Factorial modelling has been used successfully to determine nutrient requirements of commercially cultured marine fish species such as European sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and barramundi (*Lates calcarifer*). (Lupatsch et al., 1998; Lupatsch et al., 2001; Lupatsch et al., 2003; Glencross, 2008). This approach provides information for diet formulation and requirements for both growth and maintenance which are critical for formulating feeds.

Closing the life cycle of striped trumpeter provides an opportunity to study the changes in chemical, mineral and trace element composition throughout its life history. This approach is not commonplace in aquaculture research, even for established species but provides useful nutrient accretion data which can be used as a baseline to determine targets for normal growth (Shearer et al., 1994; Hernandez et al., 2003).

All of the research presented in this thesis are the first documented studies of their kind conducted on striped trumpeter larvae and post-larvae. The findings in this thesis provide valuable insight into the current status of knowledge of striped trumpeter aquaculture and lay a solid foundation for further nutrition research on this challenging but promising species. The specific objectives of each research chapter are outlined below:

1.6.1 Chapter Two: Weaning strategies for striped trumpeter (*Latris lineata*) post-larvae culture

The aims of this chapter were to determine: i) if the *Artemia* feeding stage could be shortened, ii) the optimal period of co-feeding live and microdiets, and iii) if feeding solely with microdiets was possible before 50 dph. The effect of different levels of ascorbic acid in *Artemia* enrichment on the incidence and severity of jaw deformities was also investigated.

1.6.2 Chapter Three: Effects of temperature regime on growth and development of post-larval striped trumpeter (*Latris lineata*)

The aim of this chapter was to identify the optimum temperature for rearing striped trumpeter post-larvae and to document the effects that growth rates have on development of the post-larvae into juveniles. Feed intake and whole body chemical composition were measured and a comparative slaughter technique was used to calculate utilisation efficiencies for protein and energy at the different temperatures (12, 14, 16 and 18 °C).

1.6.3 Chapter Four: The effects of ration and dietary lipid on growth of post-larval striped trumpeter (*Latris lineata*)

The aim of this chapter was to identify optimum feeding rates and rations for striped trumpeter post-larvae. Prior to this research, post-larvae were fed to satiation based on the judgment of the technician. The results from this chapter will translate into a fixed feeding schedule for striped trumpeter post-larvae reared at optimum temperatures. This was also the first attempt to measure the effects of dietary lipid inclusion on growth and development in striped trumpeter post-larvae. Feed intake was measured and a comparative slaughter technique was used to measure nutrient utilisation efficiencies. Comparisons were made between the chemical composition of post-larvae and that of juveniles.

1.6.4 *Chapter Five: Modelling nutrient requirements of post-larval striped trumpeter (Latris lineata)*

This chapter used starvation trials on different weight classes of post-larvae (1, 4, 8, 16, 32, 120 g) to supplement data from the temperature and ration and dietary lipid growth trials to develop metabolic weight exponents for protein and energy for striped trumpeter post-larvae. Factorial models were used to determine protein and energy requirements for maintenance and maximum growth. These models will have application in future work on diet formulation and nutrition research.

1.6.5 *Chapter Six: Chemical composition of striped trumpeter (Latris lineata) throughout its life-cycle*

The aim of this final research chapter was to measure the changes in chemical and elemental composition throughout the life history of striped trumpeter (from 1g to sexual maturity). Models for changes in protein, lipid, ash, moisture and energy content were developed. Elemental analysis was used to determine requirements for important elements using regression analysis. The access to striped trumpeter throughout all their life history stages made this study possible.

1.7 *Animal ethics approval*

All of the techniques and experimental procedures applied in my study were approved by the Animal Ethics Committee of the University of Tasmania. These were covered by permit number A0008719.

1.8 *Thesis structure*

Chapters two to six were written as research chapters and followed the general format of introduction, materials and methods, results and discussion. The publication status of each research chapter is detailed on the title page. Chapter two has been accepted for publication and is formatted according to the journal's requirements. References are included at the end of each chapter. There is consequently some repetition of material in the introduction, discussion and references of some of the chapters. A general discussion of the results of this study is presented as Chapter seven. Chapter seven summarises the significant findings of the research and highlights its relevance for striped trumpeter aquaculture and discusses future directions for striped trumpeter nutrition research.

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Chapter 4

The Effects of Ration and Dietary
Lipid on Growth of Post-larval Striped
Trumpeter (*Latris lineata*)

4.1 Abstract

Striped trumpeter (*Latris lineata*) is a new candidate species for adoption by the Tasmanian aquaculture industry. In preparation for future trials in sea cages, an experiment was conducted to investigate feeding strategies and the effects of dietary lipid inclusion level. Post-larval striped trumpeter ($8.1 \pm 0.1 \text{ g fish}^{-1}$) were reared using a combination of 33 %, 67 % or 100 % satiation rations and low (18 %) or high lipid (24 %) diets at a constant temperature of 15 °C. The diets were formulated to only vary nutritionally in the fish oil component. The 33 % and 67 % rations were determined by adjusting proportionally to the feed intake of fish fed to satiation (100 % ration) at the start of each week. Fish were reared for 63 d and at the end of the experiment, three fish representing post-larvae (incomplete metamorphosis) and three fish representing juveniles (complete metamorphosis) were taken from each replicate tank ($n = 4$) and measured for whole body chemical composition. The 100 % ($22.7 \pm 1.0 \text{ g}$) and 67 % ration ($21.1 \pm 1.1 \text{ g}$) produced similar weight gain; the 33 % ration ($15.4 \pm 0.7 \text{ g}$) produced significantly smaller fish at the end of the experiment. Food conversion efficiency was highest at the 33 % ration ($40.9 \pm 3.2 \%$) compared to the 67 % ($37.6 \pm 3.0 \%$) and 100 % ($30.1 \pm 2.7 \%$) rations which were similar. The 67 % (22.6 ± 0.0) and 100 % (22.7 ± 0.0) ration showed significantly higher proportions of fully metamorphosed individuals compared to the 33 % ration (8.6 ± 0.0). Dietary lipid did not have a significant effect on growth nor on food conversion efficiency. Data from starvation trials on similar sized fish reared at similar temperatures were incorporated to estimate optimum rations. The optimum ration for late stage post-larvae was found to be 4 % biomass d^{-1} . A dietary lipid content of 24 % of dry matter produced post-larvae with significantly higher carcass lipid content

(5.8 ± 0.3 % of wet weight). Post-larvae were shown to have significantly higher carcass ash content and significantly lower carcass lipid content compared to juveniles. Metamorphosis into juveniles required fish to reach a minimum weight of 23 g and a carcass lipid content of at least 4 %. Metamorphosis of the majority of post-larvae (> 50 %) was predicted to occur at weights above 20 g and carcass lipid content of 7 %. All post-larvae were predicted to have metamorphosed by 40 g. This is the first experiment to investigate the effects of ration and different dietary lipid levels on post-larval striped trumpeter metamorphosis and growth. The results emphasise the need for proper feed management to increase growth and feed efficiencies to shorten the post-larval rearing period.

4.2 Introduction

To remain competitive in the rapidly developing global seafood industry, the Tasmanian aquaculture industry is studying the striped trumpeter (*Latris lineata*) as an alternative species for aquaculture to Atlantic salmon (*Salmo salar*) (Searle and Zacharin 1994; Battaglione et al., 2008). The striped trumpeter is endemic to Tasmania and can potentially be cultured in other temperate regions. It produces firm white flesh that has high levels of polyunsaturated fatty acids (Nichols et al., 1994; Nichols et al., 2005). Research on the striped trumpeter has advanced larviculture and broodstock management to the extent that production of post-larvae is now routine (Battaglione and Cobcroft, 2007). However, the production of commercial quantities of juveniles is complicated by the prolonged neustonic, oceanic, post-larvae or „paperfish“ phase that lasts up to nine months (Furlani and Ruwald, 1999; Tracey et al., 2006; Battaglione and Cobcroft, 2007). Striped trumpeter post-larvae are susceptible to stress induced mortality and rearing under optimum conditions to increase growth is seen as a viable option to shorten the duration of the post-larval stage. Temperature has a significant effect on growth and the optimum temperature for rearing post-larvae has been determined experimentally as 14 °C (see Chapter 3).

Feeding is another important factor that has a significant influence on growth rates and performance of cultured fish species (Brett and Groves, 1979; Bureau et al., 2006; Ahmed 2007). Inadequate feed input leads to slower growth rates, delayed development and increases size variation and aggression (Carter et al., 1992; McCarthy et al., 1992; Berrill et al., 2006). Overfeeding does not produce higher growth and leads to water quality deterioration, feed wastage and organic pollution (Tsevis et al., 1992; Mihelakakis et al., 2002; Yokoyama et al., 2009). Optimising

feeding regimes and diet composition results in more efficient growth, decreased environmental impacts and increases profitability by minimising feed cost; this can account for up to 50% of variable costs in farm production (De Silva and Anderson, 1995; Lovell, 2002).

The objective of this study was to determine the optimum ration levels for post-larvae to produce the fastest growth at optimum temperatures. Two levels of dietary lipid inclusion (18% and 24%) were also tested for their effects on growth and metamorphosis. Lipid is the primary substrate for energy storage in fishes and is directly correlated to the condition and development of the fish (Weatherley and Gill, 1987; Shearer 1994). Striped trumpeter larvae have a high requirement for essential fatty acids, specifically arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3) (Bransden et al., 2005a,b). It is expected that faster growth will shorten the post-larvae stage and increase survival, ultimately lowering the production costs for striped trumpeter juveniles.

This study reared striped trumpeter post-larvae over a period of 63 d under conditions of continuous feeding and at a temperature of 14.5 °C. There was no previous data on dietary lipid inclusion for striped trumpeter post-larvae and the dietary lipid levels used are based on known dietary lipid levels for other species such as Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). The growth data was combined with studies on starvation from Chapter 5 to provide a preliminary estimate of the optimum ration for maximum growth and to investigate the effect of two dietary lipid inclusion levels on growth and performance of striped trumpeter post-larvae. Further, whole body chemical composition data for post-larvae and juveniles at the end of 63 d of

growth were used to determine the thresholds for metamorphosis (Klemetsen et al., 2003; Berrill et al., 2004; Jonsson and Jonsson, 2005).

4.3 Materials and methods

4.3.1 *Experimental diets*

Two diets which varied nutritionally only in dietary lipid content were formulated to contain low dietary lipid (LDL) or high dietary lipid (HDL) (Table 4.1). Dry ingredients were mixed with a Hobart mixer until homogenous (Fish meal, Skretting, Australia; Pre-gelatinised starch, Penford Australia Limited; Bentonite, Sigma Aldrich; α -Cellulose, Sigma Aldrich; Stay-C, Argent Laboratories; Inositol, Sigma Aldrich; Yttrium oxide, Sigma Aldrich; Carboxymethylcellulose, Sigma Aldrich; Choline chloride, Sigma Aldrich; Vitamin mix, Rabar, Queensland, Australia; Mineral mix, ingredients listed in Table 4.1; Potassium phosphate, Sigma Aldrich). Fish oil (Skretting, Australia) was then added to the diets and mixed until homogenous. Diets were cold pellet pressed to 1 mm diameter using a California Pellet Mill (CI-2 laboratory pellet mill, California Pellet Mill Co., USA). Pellets were sieved and dried at 36 °C and stored at 4 °C.

Table 4.1. Ingredient and proximate composition of the experimental diets

Ingredient composition (g kg ⁻¹)	Low dietary lipid	High dietary lipid
Fish meal	690.00	690.00
Fish oil	90.00	140.00
Pre-gelatinised starch	100.00	100.00
Bentonite	44.50	14.50
α -Cellulose	40.00	20.00
Carboxymethylcellulose	10.00	10.00
Vitamin mix	3.00	3.00
Mineral mix ^a	5.00	5.00
Potassium phosphate (KH ₂ PO ₄)	10.00	10.00
Stay-C	3.00	3.00
Choline chloride	2.00	2.00
Inositol	0.50	0.50
Yttrium oxide	2.00	2.00
Chemical composition (g kg ⁻¹ dry matter)		
Dry matter (g kg ⁻¹)	893.20	890.41
Crude protein	442.50	434.69
Total lipid	187.49	237.25
Ash	179.11	149.88
Gross energy (MJ g ⁻¹)	19.47	21.21

^a Mineral mixture (mg kg⁻¹ mixture): α -Cellulose 612.3; Fe 544.7 (FeSO₄.7H₂O); Zn 197.9 (ZnSO₄.7H₂O); Mn 92.3 (MnSO₄.7H₂O); Cu 35.4 (CuSO₄ anhydrous); Co 14.3 (CoSO₄.6H₂O); I 2.2 (KI); Se 1.0 (Na₂SeO₃); all mineral premix ingredients were obtained from Sigma Aldrich.

4.3.2 *Source of animals and experimental system*

Larvae were hatched from eggs originating from captive broodstock and were subsequently reared using established hatchery protocols (Battaglione and Cobcroft, 2007). The experimental system at the Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute (MRL) consisted of twenty-four 150 L round flat-bottomed tanks with marbled sides and black bottoms and external stand pipes. Over each tank was an automatic feeding unit (Curnow et al. 2006). Flow rates into each tank were maintained at 162 L h^{-1} . Lighting was provided by fluorescent globes, light intensity measured from the water surface was of $2.8 \pm 0.1 \mu\text{mol}$. Photoperiod was maintained at 16h light : 8h dark. Incoming seawater was filtered through one micron bag filters and passed through heat chillers. Temperature was maintained at $14.5 \pm 0.4^\circ\text{C}$. Water quality was measured at 10:00 am daily using a YSI 660 Multi-probe (YSI Incorporated, USA) for temperature, pH and salinity and an Oxyguard™ Handy Polaris probe (Oxyguard International, Denmark) for oxygen. Water quality parameters were: pH, 8.1 ± 0.1 , salinity, $33.9 \pm 0.2 \text{ ppt}$, oxygen saturation, $97.9 \pm 2.59 \%$. Faeces and uneaten food were removed daily from the tanks using a siphon and 70 % water replaced every afternoon to maintain high water quality.

4.3.3 *Experimental design*

At 287 days post-hatch (dph), 20 striped trumpeter post-larvae ($8.1 \pm 0.1\text{g}$, mean \pm SE, $n = 480$) were randomly stocked into each of the experimental tanks. Fish that had severe jaw malformations which hindered their capacity to feed were not used. Twenty five fish were euthanased using Aqui-S™ (Aqui-S, New Zealand)

at a dose of $10\ 200^{-1}\ \text{v}\ \text{v}^{-1}$ Aqui-S™ in seawater for assessment of initial whole-body chemical composition. Fish in tanks were acclimated for a period of 24 d and fed with a 50:50 mix of the LDL and HDL diets at a fixed ration of $10\ \text{g}\ \text{d}^{-1}$. Feeders were programmed to dispense feed equally every hour throughout the 16 h light period. Mortalities during acclimation were replaced with fish from the mass rearing tank.

At the beginning of the experiment, fish were anaesthetised using Aqui-S™ (Aqui-S, New Zealand) at a dose of $1\ 200^{-1}\ \text{v}\ \text{v}^{-1}$ seawater and individually measured for wet weight (WW) to 0.1 g and total length (TL) to 1 mm. Each tank was randomly assigned a ration of 33, 67 or 100% of satiation and either the LDL or HDL diet in a fully orthogonal design. At the beginning of each week, the 100% ration tanks were hand fed their respective diets to satiation every hour throughout the light period. Satiation was determined to be reached when approximately five pellets were left on the tank bottom. Feed intake (FI) was calculated by subtracting the uneaten pellets from the amount of food fed. The 67% and 33% rations were calculated weekly from the feed intake of the 100% ration groups. Fish were anaesthetised and weighed (to 0.1 g) and measured (to 1 mm) individually every 21 d. Fish were scored for development using the characteristic appearance of lateral stripes scored on a scale of zero to three, with zero indicating the absence of stripes and three indicating complete development into juveniles (see Chapter 3). At the end of the experiment, three post-larvae and three metamorphosed post-larvae were taken randomly from each tank, euthanased and frozen. Whole fish were autoclaved (Williams et al., 1995) and freeze dried to a constant weight prior to whole-body chemical composition analysis (see 4.3.4).

4.3.4 Chemical Analysis

Samples of diets were freeze dried to constant weight and ground to a homogenous powder. Standard methods were used to determine dry weight (freeze-drying to a constant weight); crude protein (Thermo Finnigan 1112 Series elemental analyser, $N \times 6.25$); total lipid (Bligh and Dyer, 1959); ash by combustion at 550 °C for 6 h (AOAC, 1995). Energy content of the diets was determined by bomb calorimetry (AOAC, 1995). Three replicate measurements for crude protein, total lipid and ash were performed for each fish. All composition data are presented as a mean percentage of the WW of each individual fish sampled from each treatment (Shearer et al., 1994).

4.3.5 Calculations

Condition factor (k) was calculated using the formula $k = (WW/TL^3) \times 100$ where WW is wet weight and TL is total length (Weatherley and Gill, 1987). Specific growth rate (SGR, % day⁻¹) was calculated from mean WW using the formula: $SGR = 100 \times (\ln \text{final mean WW} - \ln \text{initial mean WW}) / D$; where D is the number of days. Coefficient of variation for WW per tank (CV, %) was calculated as: $100 \times SD / \text{mean WW}$. Feeding rate was expressed a percentage of biomass (FR, % BM d⁻¹) and was calculated using the formula: $FR = 100 \times (\text{total feed input} / D) / ((\text{initial biomass} + \text{final biomass}) / 2)$; where D is the number of days. Food conversion efficiency (FCE) was calculated by dividing the total gain in biomass by the total food consumed for each growth period. The total food consumed was adjusted to account for food consumed by mortalities on a pro rata basis. Energy

content of whole fish was calculated by using the conversion factors of Brafield (1985) for protein (23.6 KJ g^{-1}) and lipids (36.2 KJ g^{-1}). Productive protein value (PPV, %) was calculated using the formula: $\text{PPV} = 100 \times (\text{Fish protein gain (g)} / \text{Total protein consumed (g)})$. Productive lipid value (PLV, %) was calculated using the formula: $\text{PLV} = 100 \times (\text{Fish lipid gain (g)} / \text{Total lipid consumed (g)})$. Productive energy value (PEV, %) was calculated using the formula: $\text{PEV} = 100 \times (\text{Fish energy gain (kJ)} / \text{Total energy consumed (kJ)})$.

4.3.6 Statistical analysis

All results are reported as mean \pm SE. Data were tested for normality and homogeneity using Levene's test of equality. Percent data were arcsine square-root-transformed prior to analysis. Univariate ANOVA with ration and dietary lipid as the two fixed factors was used to detect differences in survival, mean WW, mean TL, condition (k), coefficient of variation of WW, SGR, metamorphosis success, FCE, PPV, PLV, and PEV. Univariate ANOVA with ration, dietary lipid content and life history stage (post-larvae or juveniles) as the fixed factors was used to analyse differences in whole carcass chemical composition. Samples were pooled according to ration when dietary lipid was not found to have a significant effect ($p > 0.25$). When significant interactions were found, a univariate ANOVA was used to detect differences between the groups. A Tukey's post-hoc multiple comparison test was used to separate means. Statistical significance was accepted at $p \leq 0.05$. A logistic curve model was used to estimate the proportion of fully metamorphosed individuals at a given weight class fed the three rations. Statistical analyses were performed

using SPSS version 15.0 statistical software. Quadratic polynomial and logistic curves were fitted using SigmaPlot 9.0.

Data from starvation trials in Chapter 5 were used in estimating optimum ration using quadratic polynomial curves. Logistic curves were fitted to the proportion of metamorphosed individuals using wet weight and carcass lipid content as categories. SigmaPlot version 11.0 was used to formulate best fit curves.

4.4. Results

4.4.1 *Survival*

There were no significant differences in survival attributable to ration ($df = 2$, $F = 1.14$, $p = 0.34$, $n = 8$), dietary lipid ($df = 1$, $F = 0.33$, $p = 0.58$, $n = 12$), nor was there any interaction between ration and dietary lipid ($df = 2$, $F = 0.08$, $p = 0.92$, $n = 4$). Survival was high and the overall mean survival rate was 95.6 ± 2.3 %.

4.4.2 *Growth and development*

There were no significant differences in the mean WW between treatments at the beginning of the experiment ($df = 2$, $F = 1.37$, $p = 0.28$, $n = 8$). There was no significant difference in growth and development with dietary lipid content at 21 d ($df = 1$, $F = 1.6$, $p = 0.22$, $n = 12$); 42 d ($df = 1$, $F = 0.5$, $p = 0.50$, $n = 12$) and 63 d ($df = 1$, $F = 0.1$, $p = 0.93$, $n = 12$). There was no significant interaction between ration and dietary lipid content at 21 d ($df = 2$, $F = 0.7$, $p = 0.50$, $n = 4$); 42 d ($df = 1$, $F = 1.1$, $p = 0.37$, $n = 4$) and 63 d ($df = 1$, $F = 1.2$, $p = 0.33$, $n = 4$). Ration was found

to have a significant effect on mean WW, mean TL, condition (k), SGR and success of metamorphosis. Fish fed with 33 % ration were significantly smaller than fish fed with 67 % and 100 % rations, which had reported similar mean WW, at 21 d ($df = 2$, $F = 14.0$, $p < 0.001$) at 42 d ($df = 2$, $F = 18.2$, $p < 0.001$) and at 63 d ($df = 2$, $F = 16.3$, $p < 0.001$) (Fig. 4.1). There were no significant differences in the coefficient of variation of WW of individual fish in any sampling period.

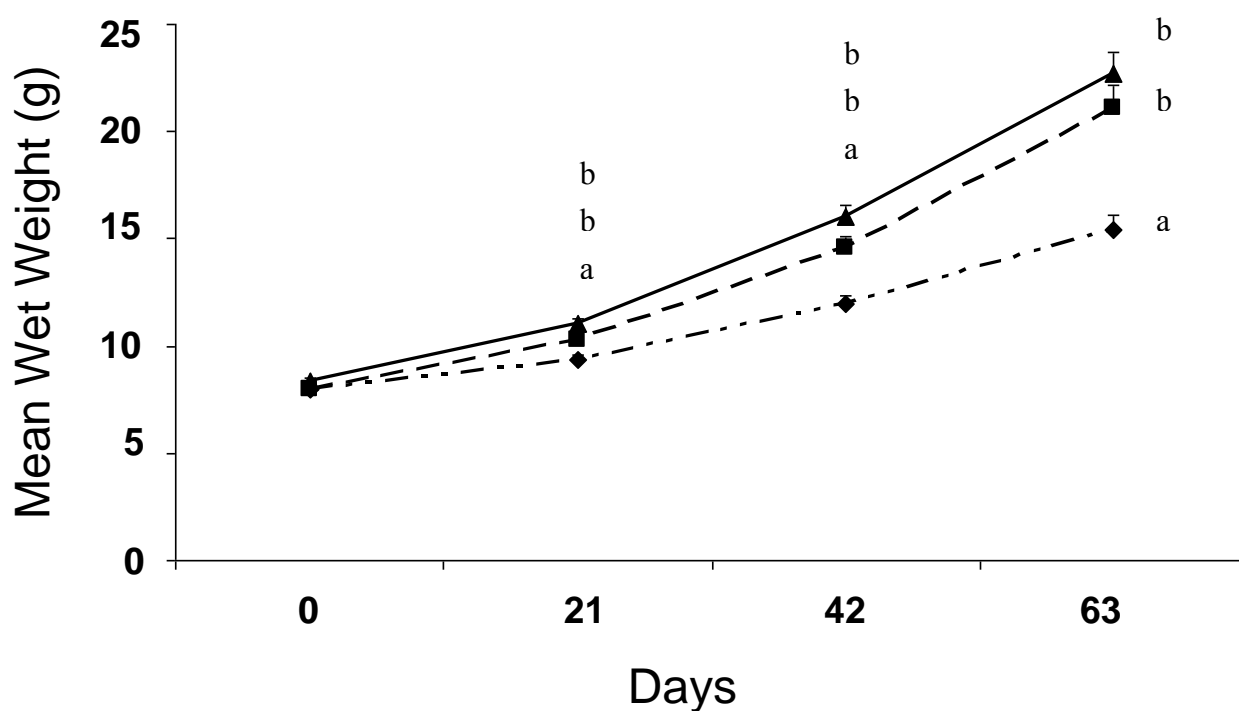


Figure 4.1: Growth (mean WW \pm SE) of striped trumpeter post-larvae fed three different levels of ration ($\diamond = 33\%$, $\blacksquare = 67\%$, $\blacktriangle = 100\%$) over 63 d. Significant differences in wet weight are represented using different letters at each 21 d measurement ($p \leq 0.05$).

Mean TL of fish showed similar trends to mean WW with ration producing significant differences in mean TL but not dietary lipid. At 21 d, fish fed the 100 % ration were significantly longer than fish fed with 33 % ration ($df = 2$, $F = 7.1$, $p = 0.005$). Fish fed with 33 % ration were significantly shorter than fish fed with 67 % and 100 % rations, which had similar mean TL, at 42 d ($df = 2$, $F = 14.0$, $p < 0.001$) and at 63 d ($df = 2$, $F = 14.1$, $p < 0.001$) (Table 4.2).

At 21 d, mean condition (k) of fish fed with 33 % ration was significantly lower than fish fed with 67 % ration, fish fed with 100 % ration had the highest condition (k) ($df = 2$, $F = 20.7$, $p < 0.001$). Fish fed with 33 % ration showed the lowest mean condition (k) compared to the 67 % and 100 % ration fed fish, which had similar mean condition (k), at 42 d ($df = 2$, $F = 7.7$, $p = 0.003$) and at 63 d ($df = 2$, $F = 7.4$, $p = 0.005$) (Table 4.2).

Table 4.2: Mean total length and condition k (mean \pm SE) of striped trumpeter post-larvae fed with different rations. Within sampling periods, treatments not sharing the same letter are significantly different ($p \leq 0.05$).

Mean TL (mm)	33 % Ration	67 % Ration	100 % Ration
21 d	112.1 \pm 2.7 ^a	114.0 \pm 3.0 ^{ab}	116.2 \pm 2.8 ^b
42 d	118.3 \pm 3.1 ^a	123.3 \pm 3.5 ^b	126.6 \pm 3.4 ^b
63 d	125.1 \pm 3.6 ^a	133.5 \pm 4.3 ^b	137.1 \pm 4.1 ^b
Condition (k)			
21 d	0.6 \pm 0.0 ^a	0.7 \pm 0.0 ^b	0.7 \pm 0.0 ^c
42 d	0.7 \pm 0.0 ^a	0.7 \pm 0.0 ^b	0.7 \pm 0.0 ^b
63 d	0.7 \pm 0.0 ^a	0.8 \pm 0.0 ^b	0.8 \pm 0.0 ^b

The SGR of fish fed with 33 % ration was significantly lower than fish fed with 67 % and 100 % rations, which were similar, after 21 d ($df = 2$, $F = 18.2$, $p < 0.001$), 41 d ($df = 2$, $F = 17.3$, $p < 0.001$) and 63 d ($df = 2$, $F = 7.1$, $p = 0.005$).

When SGR was calculated for the whole growth period, fish fed 33 % ration showed significantly lower SGR compared to 67 % and 100 % ration fed groups which showed similar SGR ($df = 2$, $F = 17.6$, $p < 0.001$) (Table 4.3).

Table 4.3: Mean specific growth rate (SGR; mean \pm SE) of striped trumpeter post-larvae fed with different rations. Within sampling periods, treatments not sharing the same superscript are significantly different ($p \leq 0.05$).

Mean SGR (% d ⁻¹)	33 % Ration	67 % Ration	100 % Ration
0-21 d	0.7 \pm 0.1 ^a	1.2 \pm 0.1 ^b	1.4 \pm 0.1 ^b
22-42 d	1.2 \pm 0.1 ^a	1.7 \pm 0.1 ^b	1.8 \pm 0.1 ^b
43-63 d	1.2 \pm 0.1 ^a	1.7 \pm 0.1 ^b	1.6 \pm 0.1 ^b
0-63 d	1.0 \pm 0.1 ^a	1.5 \pm 0.1 ^b	1.6 \pm 0.1 ^b

Groups fed with 67 % (22.6 ± 0.0) and 100 % (22.7 ± 0.0) ration showed significantly higher proportions of fully metamorphosed individuals compared to the 33 % ration (8.6 ± 0.0) groups ($df = 2$, $F = 6.0$, $p = 0.01$).

4.4.3. Feed intake and food conversion efficiency

Measurements of feed intake are confounded by the behaviour of the fish which sometimes chewed the pellet and regurgitated it out through the mouth and gill openings. Pellets which were regurgitated appeared as fine particles on the tank bottom and were missed during counting of leftover pellets. Feed intake measurements are overestimated because of this phenomenon. By controlling the feed input, total feed fed to the 33, 67 and 100 % ration groups was significantly different at 21 d ($df = 2$, $F = 284.26$, $p < 0.001$), at 42 d ($df = 2$, $F = 159.84$, $p < 0.001$), at 63 d ($df = 2$, $F = 43.56$, $p < 0.001$) and for the entire experimental period

($df = 2$, $F = 68.46$, $p < 0.001$). The LDL and HDL diets were fed at the same rate according to the ration treatment and were not significantly different. Fish fed 100 % ration consumed more food during the first 42 d of the experiment regardless of diet fed ($df = 2$, $F = 12.8$, $p < 0.001$). No significant differences were found in the feeding rates for the 67 % ration ($df = 2$, $F = 2.8$, $p = 0.09$). A significantly higher amount of food was provided to the 33 % ration treatment from 0 to 21 d compared to 22 to 63 d ($df = 2$, $F = 5.9$, $p = 0.01$). Mean feeding rates for each 21 d period and the entire experimental period are summarised in Table 4.4.

Table 4.4: Mean feeding rates per ration treatment (% BM d⁻¹; mean \pm SE). Treatment rations were significantly different and are noted with different letters. For each ration, feeding periods not sharing the same superscript are significantly different ($p \leq 0.05$).

Mean Feeding Rate	33 % Ration ^a	67 % Ration ^b	100 % Ration ^c
0-21 d	2.2 \pm 0.1 ^a	4.0 \pm 0.1	5.4 \pm 0.1 ^b
22-42 d	2.5 \pm 0.1 ^b	4.4 \pm 0.1	5.2 \pm 0.1 ^b
43-63 d	2.6 \pm 0.1 ^b	3.9 \pm 0.2	4.5 \pm 0.2 ^a
0-63 d	2.4 \pm 0.1	3.8 \pm 0.1	4.7 \pm 0.2

Fish fed with 33 % ration showed significantly higher FCE compared to fish fed with 100 % ration for the period from the beginning of the experiment to 21 d ($df = 2$, $F = 6.0$, $p = 0.010$) and from 21 d to 42 d ($df = 2$, $F = 4.8$, $p = 0.022$). There were no significant differences in FCE between 33 %, 67 % and 100 % ration fed groups for the period from 42 d to 63 d. There were no significant differences over the entire growth period in FCE between the three rations (Table 4.5).

Table 4.5: Mean food conversion efficiency (FCE; mean \pm SE) of striped trumpeter post-larvae fed with different rations. Within sampling periods, treatments not sharing the same superscript are significantly different ($p \leq 0.05$).

Mean FCE (%)	33 % Ration	67 % Ration	100 % Ration
0-21 d	32.4 \pm 2.8 ^b	29.6 \pm 2.5 ^{ab}	22.2 \pm 1.3 ^a
22-42 d	44.2 \pm 2.7 ^b	38.2 \pm 2.7 ^{ab}	31.9 \pm 3.2 ^a
43-63 d	42.9 \pm 4.8	41.4 \pm 4.4	33.5 \pm 3.5
0-63 d	40.9 \pm 3.2	37.6 \pm 3.0	30.1 \pm 2.7

4.4.4 Chemical composition

Ideally three post-larvae (not metamorphosed) and three juveniles (metamorphosed) were sampled from each tank. However, some tanks did not show a high degree of metamorphosis, therefore fish closest to juveniles in appearance were sampled resulting in an unbalanced number of post-larvae ($n = 88$) and juveniles ($n = 52$). The chemical composition of post-larvae and juveniles were compared to detect any differences protein, total lipid, ash energy and moisture content.

4.4.4.1 Moisture

The moisture content (% WW; mean \pm SE) of 100 % ration fed tanks (72.6 ± 0.3 %) was significantly lower than 33 % ration groups (74.4 ± 0.4 %) ($df = 2$, $F = 6.27$, $p = 0.003$). Moisture content of juveniles (72.5 ± 0.3 %) was significantly lower than the moisture content of post-larvae (74.1 ± 0.2 %) ($df = 1$, $F = 16.02$, $p < 0.001$). A significant interaction between ration and diet was found; groups fed a 100 % ration with HDL diet showed significantly lower moisture content ($70.8 \pm$

0.6 %) compared to groups fed 33 % rations with LDL (75.0 ± 0.7 %) and HDL diets (74.4 ± 0.4 %) ($df = 2$, $F = 4.27$, $p = 0.016$).

4.4.4.2 Ash

The ash content (% WW; mean \pm SE) of 100 % ration groups (3.5 ± 0.0 %) was significantly lower than the ash content of the 33 % ration groups (3.8 ± 0.1 %) ($df = 2$, $F = 5.79$, $p = 0.004$). A significant interaction between ration and life history stage was found; juveniles (3.6 ± 0.1 %) and post-larvae (3.5 ± 0.1 %) from 100 % rations groups and juveniles (3.6 ± 0.1 %) from 33 % ration groups had significantly lower ash content compared to post-larvae (3.9 ± 0.1 %) from 33 % ration groups ($df = 2$, $F = 4.77$, $p = 0.010$). A significant interaction between ration level, dietary lipid level and life history stage was also found; post-larvae from 100 % ration groups fed with the LDL diet (3.4 ± 0.1 %) and juveniles fed a 33 % ration with the LDL diet (3.4 ± 0.1 %) had significantly lower ash content compared to post-larvae fed a 33 % ration with the LDL (3.9 ± 0.1 %) and HDL diets (3.9 ± 0.1 %) ($df = 2$, $F = 3.77$, $p = 0.026$).

4.4.4.3 Crude protein

No significant differences in crude protein content were found attributable to the different ration and dietary lipid level treatments and life history stages. There were also no significant interactions found between the fixed factors that influenced crude protein content. Overall mean crude protein content was 16.9 ± 0.0 %.

4.4.4.4 *Total lipid*

Total lipid content of fish from 33 % ration groups (3.8 ± 0.3 %) was significantly lower compared to fish from the 67 % (5.5 ± 0.4 %) and 100 % ration groups (6.0 ± 0.4 %) ($df = 2$, $F = 12.56$, $p < 0.001$). Fish fed with the LDL diet (4.5 ± 0.3 %) had significantly lower total lipid content compared to fish fed with the HDL diet (5.8 ± 0.3 %) ($df = 1$, $F = 12.90$, $p < 0.001$). Lipid content of post-larvae (4.0 ± 0.2 %) were significantly lower than total lipid content in juveniles (7.0 ± 0.3 %) ($df = 1$, $F = 61.78$, $p < 0.001$).

4.4.5 *Nutrient retention efficiency*

Fish fed with 33 % ration (18.3 ± 1.3 %) had significantly higher PPV compared to the 67 % (15.6 ± 0.9 %) and 100 % ration groups (13.3 ± 1.0 %) ($df = 2$, $F = 6.4$, $p = 0.008$). Ration did not influence PEV and PLV. Overall mean PEV was 15.1 ± 0.7 % and overall mean PLV was 14.4 ± 0.8 %. Dietary lipid content and the interaction between ration and dietary lipid did not influence PPV, PEV and PLV.

4.4.6 *Estimation of optimum ration*

Data for loss of weight and depletion of body nutrient reserves during starvation was determined from a separate series of experiments where fish of different size classes were starved in individual tanks for a period of 10 d (see Chapter 5). Data obtained from 16 g and 32 g fish starved at 14 °C and 16 °C ($n = 4$) were determined to be closest to the sizes of fish and rearing conditions of this study.

Optimum rations for growth expressed in gains of biomass, mass of protein, mass of lipid and energy content were calculated using fitted quadratic polynomial curves. The equations of the curves are summarized in Table 4.6. Predicted maximum rations for SGR, SGR Protein, SGR Lipid and SGR Energy were 4.2 %, 4.1 %, 4.2 % and 4.2 %, respectively (Fig. 4.2 and 4.3).

A calculated maintenance ration of 0.9 % BM d⁻¹ was found using the SGR curve. This was combined with the FCE data for individual tanks over the 63 d experimental period to provide a predictive model for estimating optimal rations. Maximum FCE was predicted to be achieved with a ration of 3.2 % BM d⁻¹ ($r^2 = 0.550$, $F_{2,22} = 13.45$, $p = 0.0002$) (Fig. 4.4).

Table 4.6: Quadratic polynomial equations describing best-fit lines for SGR, SGR Protein, SGR Lipid and SGR Energy at different rations; where R represents ration (% BM d⁻¹).

	$y = a + bR + cR^2$			r^2	F _{2,25}	p
	A	b	c			
SGR (% d ⁻¹)	-1.007	1.241	-0.148	0.941	197.602	<0.0001
SGR Protein (% d ⁻¹)	-1.175	1.340	-0.165	0.924	152.013	<0.0001
SGR Lipid (% d ⁻¹)	-3.240	3.203	-0.385	0.923	149.293	<0.0001
SGR Energy (% d ⁻¹)	-1.346	1.586	-0.190	0.952	246.925	<0.0001

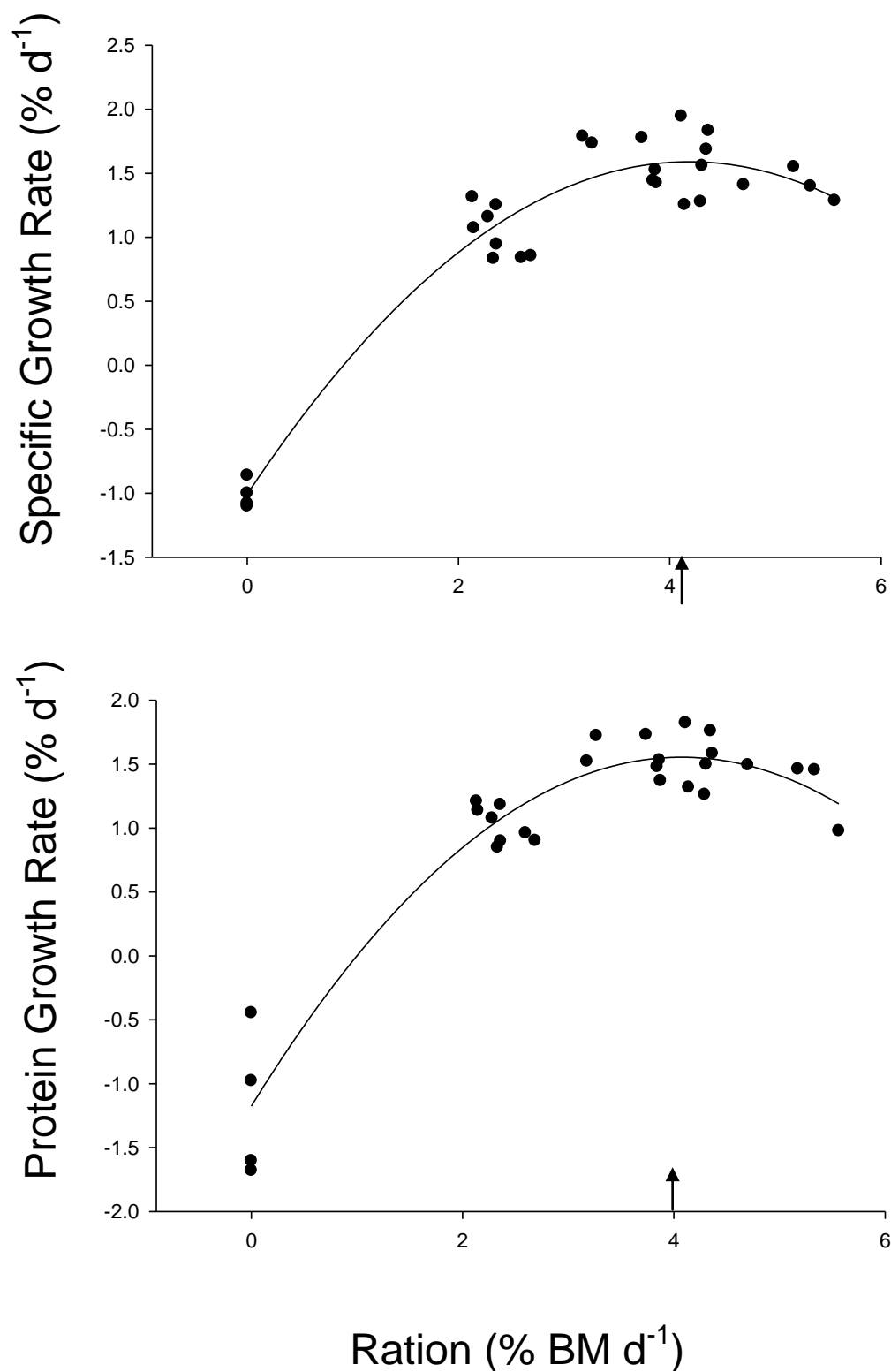


Figure 4.2: Relationship between specific growth rate (% d⁻¹) and specific protein growth rate (% d⁻¹) and ration (% BM d⁻¹) described with a quadratic polynomial curve. Arrows indicate predicted maximum.

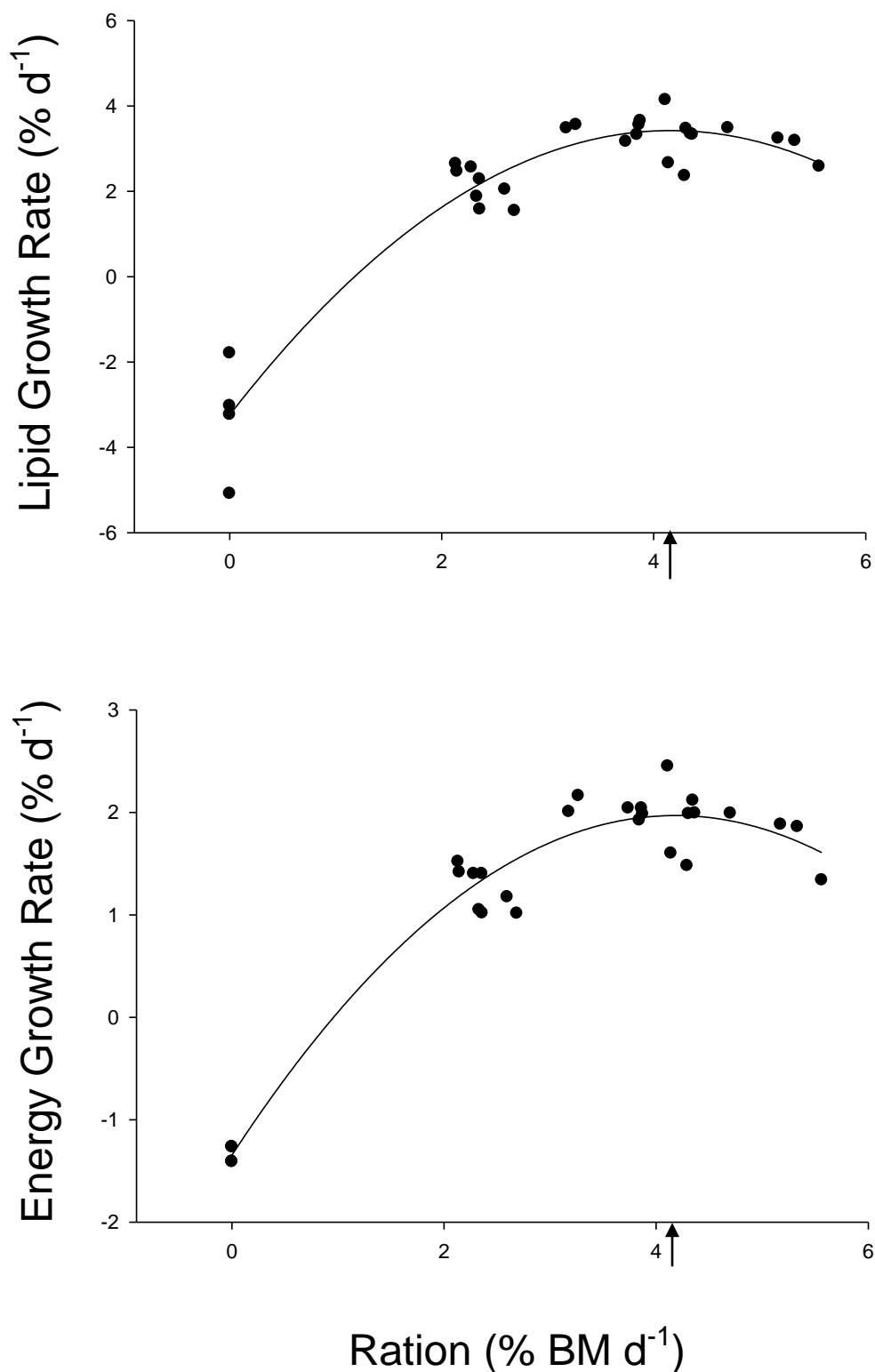


Figure 4.3: Relationship between specific lipid growth rate (% d⁻¹) and specific energy growth rate (% d⁻¹) and ration (% BM d⁻¹) described with a quadratic polynomial curve. Arrows indicate predicted maximum.

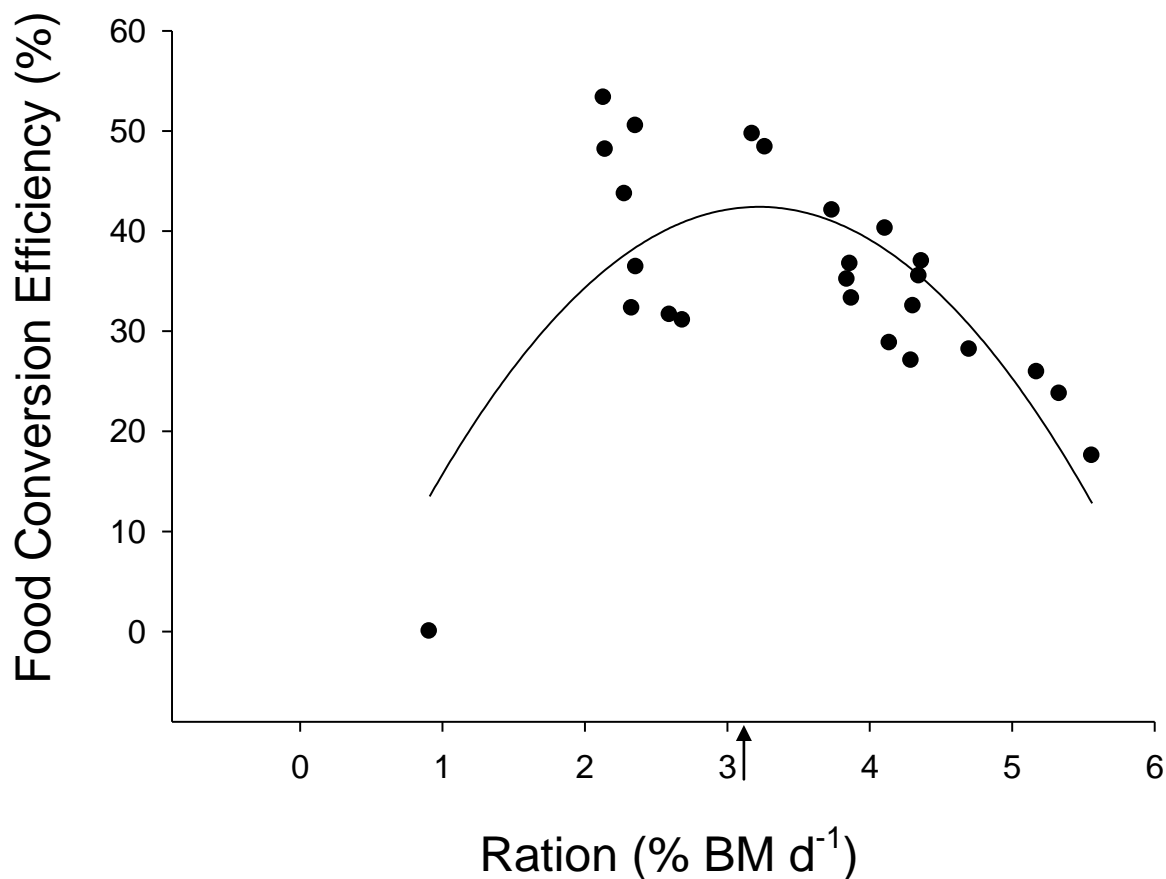


Figure 4.4: Relationship between food conversion efficiency (%) and ration (% BM d⁻¹) described with a quadratic polynomial curve with the equation $y = -13.74 + 34.86 X R - 5.41 X R^2$; where R is ration (% BM d⁻¹). Arrows indicate predicted maximum.

4.4.7. Estimation of thresholds for metamorphosis into juveniles

It was noted during weekly measurements that the progression of metamorphosis of individuals of similar sizes varied. Examples of fish of the same size but of different life history stages are presented (Fig. 4.5). Data from the 67 % and 100 % ration treatments were pooled based on previous ANOVA analysis for both wet weight and carcass lipid content. The proportion of metamorphosed fish from the 33 % ration treatment were compared to the proportion of metamorphosed fish from the pooled 67 % and 100 % ration treatments according to wet weight (Fig. 4.6). Fish from the 67 % and 100 % ration treatments showed a higher proportion of metamorphosed individuals from 20 to 35 g. At 40 g all three ration treatments showed complete metamorphosis. Data for sizes beyond 40 g for the 33 % ration treatment were not available and it was assumed that fish would show complete metamorphosis beyond this size.

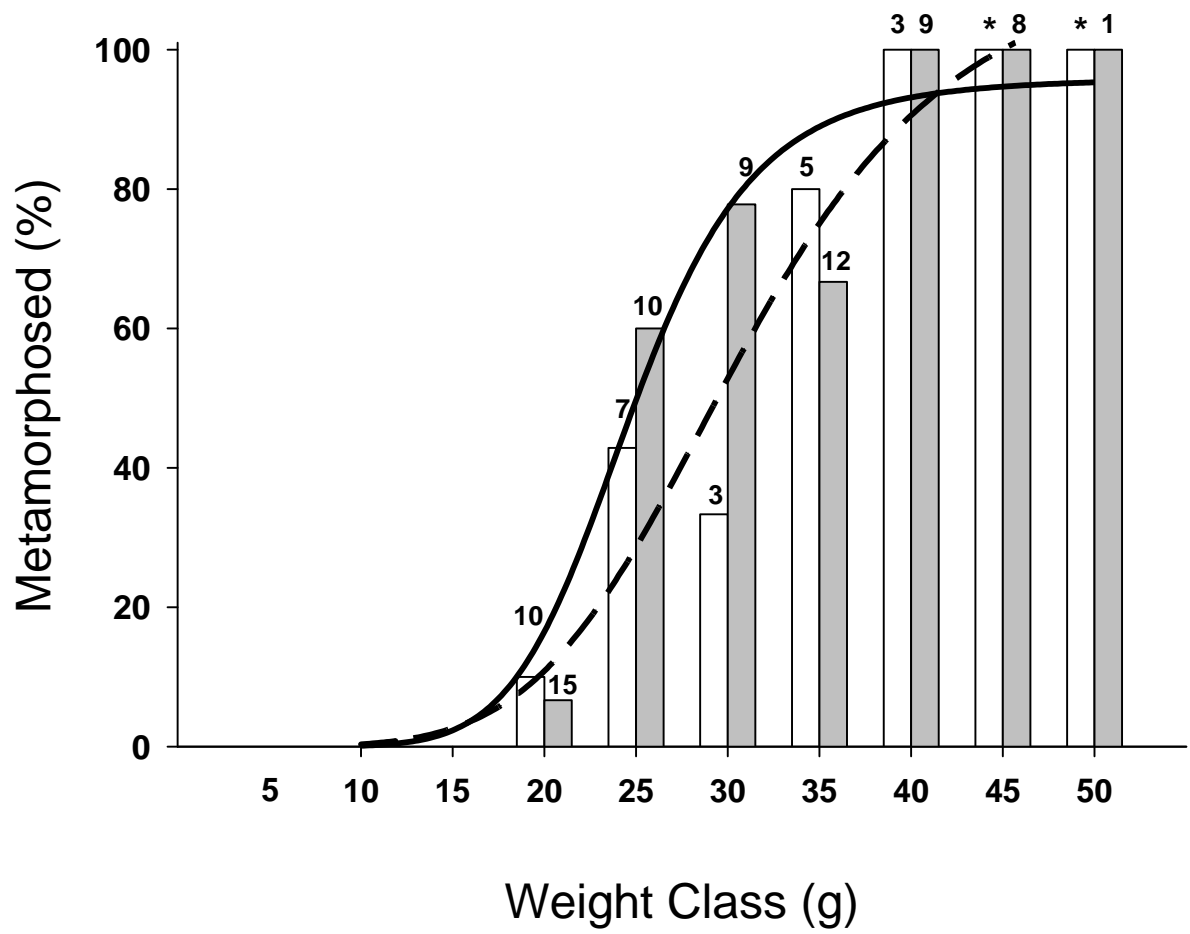


Figure 4.5: Photograph of specimens of similar sizes showing different life history stages; the fish on top shows signs of metamorphosing into the juvenile form while the bottom fish is still a post-larvae in appearance.

The proportion of metamorphosed fish from the 33 % and the pooled 67 % and 100 % ration treatments were compared according to carcass lipid content (Fig. 4.7). The carcass lipid content of fish from the 33 % ration treatment reached a maximum of 7 % and did not reach the point at which complete metamorphosis could be expected. Complete metamorphosis of fish from the 67 % and 100 % ration treatment occurred at 11 % carcass lipid content. Equations for the logistic curves are summarised in Table 4.7.

Table 4.7: Equations for fitted logistic curves describing best-fit for metamorphosed individuals according to weight class (g) and carcass lipid content (%).

	$y = a / 1+(x / x^0)^b$			r^2	F	p
	a	b	x^0			
Weight class						
33 % ration	114.3	-5.2	30.9	0.95	80.8	<0.001
67 % and 100 % ration	95.8	-7.4	24.7	0.96	75.2	<0.001
Carcass lipid content						
33 % ration	90.7	-3.5	5.1	0.89	15.8	0.012
67 % and 100 % ration	129.6	-3.0	8.0	0.86	31.4	0.0002



the 33 % ration. Logistic curves for the 33 % ration treatment (hatched line) and the pooled 67 % and 100 % (solid line) treatments.

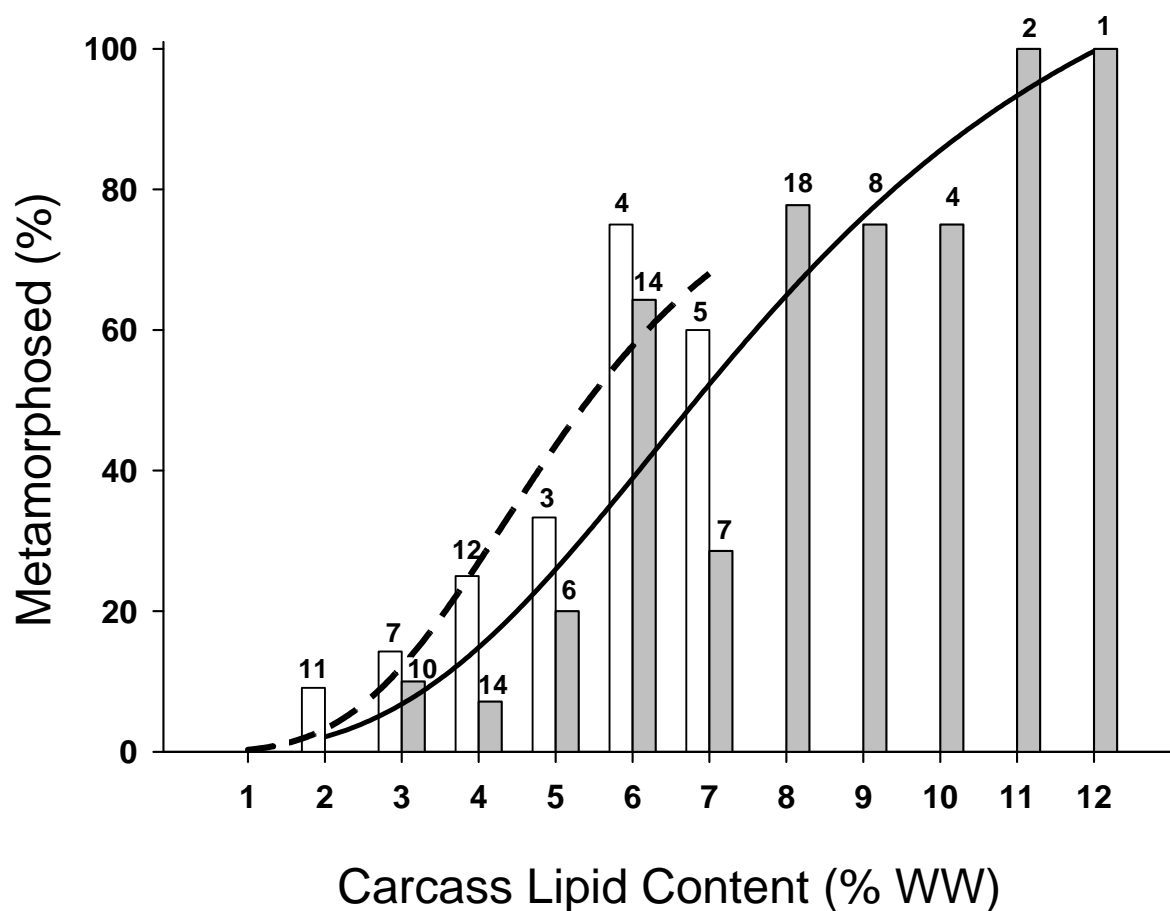


Figure 4.7: Proportion of metamorphosed individuals grouped according to carcass lipid content from the 33 % ration treatment (□) and the pooled 67 % and 100 % treatments (■). The number of fish in each categorical class is indicated above the respective bars. Logistic curves for the 33 % ration treatment (hatched line) and the pooled 67 % and 100 % (solid line) treatments.

The whole population was sampled at 63 d and individual wet weights plotted against carcass lipid content. Majority (> 95 %) of the post-larvae population weighed between 6 g and 20 g. Metamorphosed individuals were first observed at 19 g. Majority (> 95 %) of the metamorphosed individuals weighed from 23 g upwards and had a carcass lipid content of 4 % upwards (Fig. 4.8).

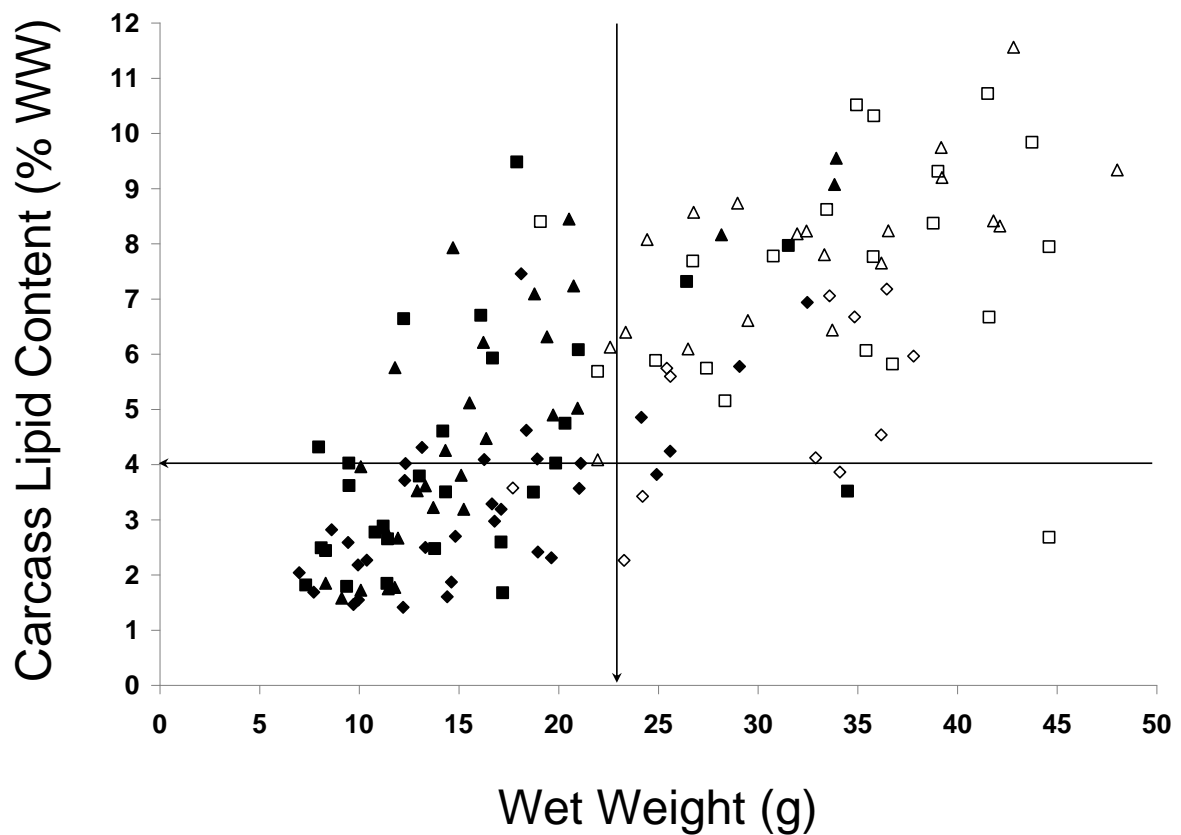


Figure 4.8: Relationship between wet weight and carcass lipid content of post-larvae and juvenile striped trumpeter. Solid data markers represent post-larvae and hollow data markers represent juveniles from the 33 % ration (\diamond), the 67 % ration (\square) and 100 % ration (\triangle) treatments. Arrows indicate the point where majority (> 95 %, $n = 140$) of the metamorphosed population is found.

4.5 Discussion

4.5.1 *Growth and identifying the optimum ration*

The current study expands our understanding of optimal culture conditions for striped trumpeter post-larvae by investigating the effects of feeding, specifically ration and dietary lipid content, on growth and other indicators of performance. Prior to this study, there were no empirical studies on feeding rates for post-larval striped trumpeter. This study quantified the relationship between feeding rate and growth rate for post-larval striped trumpeter. Many other studies have shown that as ration increases, wet weight growth increases up to an optimum point and then decreases at rations beyond the optimum (Brett and Groves, 1979; Weatherley and Gill, 1987; Jobling, 1994). Feeding 33 % of satiation, which translated to 2.4 % BM⁻¹ actual feed intake, resulted in the lowest growth. Growth of fish fed at 67 % of satiation and to satiation, which translated to 3.8 % and 4.7 % BM d⁻¹, produced equally high growth rates. Therefore, increasing feed intake from 3.8 to 4.7 % BM d⁻¹ did not result in an incremental increase in wet weight growth. These relationships were also found for other measures of growth and performance, namely, total length, condition (*k*) and SGR. These findings indicated that the optimum ration lies between 3.8 and 4.7 % BM d⁻¹. The optimum ration found in this study was determined under continuous feeding and at 14.5 °C. A change in culture conditions or feeding regimes will require identification of an optimal ration under those conditions.

The chemical composition of wet weight growth was also affected by ration. Feeding below the optimum range resulted in lower total lipid and higher ash and moisture content. Protein content of the carcass did not differ between treatments.

These findings agree with those of Shearer (1994) and Bureau et al., (2006) which showed an inverse relationship between body lipid and moisture and that protein content in the carcass remained stable as weight increases. Further, protein content was not affected by growth rate, diet or environment. The higher ash content found in the group fed below the optimum ration reflected the lower condition of fish from those treatments.

Fish fed with the HDL diet (24 %) showed higher total lipid which indicated a higher accumulation of energy reserves (Jobling, 2001). This increase in reserves did not result in higher wet weight growth compared to fish fed with the LDL diet but has implications for health and husbandry if fish with higher energetic reserves are less susceptible to stress induced mortality. In future work, it will also be useful to identify the optimum dietary lipid inclusion rates and the potential replacement of fish oil with alternative oils in the face of increasing pressure on worldwide fish oil supplies (Miller et al., 2008).

To identify the optimum ration, we combined the collected growth data with results from separate starvation trials on similar sized fish held for 10 days at 14 °C and 16 °C (see Chapter 5). Polynomial curves fitted to SGR, SGR-Protein, SGR-Lipid, SGR-Energy and FCE all indicate that the optimum ration is 4.0 % BM d⁻¹. Feeding above this level led to overfeeding and lower feed conversion efficiency. Other studies have also found this effect of overfeeding on growth and conversion efficiency (Tsevis et al., 1992; Mihelakakis et al., 2002; Fiogbe and Kestemont, 2003; Wang et al., 2007).

The observed growth efficiency of striped trumpeter post-larvae was lower compared to results from other research studies on cultured species. The highest FCE achieved in this study was 41 %. Studies on other species have found higher FCE

such as those on European sea bass (*Dicentrarchus labrax*) with an FCE of 90 % (Eroldogan et al., 2004) and an FCE of 75 % (Peres and Oliva-Teles, 2005); a study on barramundi (*Lates calcarifer*) reported an FCE of 146 % (Katersky and Crater, 2005); and a study on Atlantic salmon (*Salmo salar*) parr was able to achieve an FCE of 144 % (Bendiksen et al., 2003).

Likewise the highest PPV (18 %) and PEV (15 %) values of striped trumpeter post-larvae were much lower compared to species such as barramundi (*Lates calcarifer*) which had a PPV of 50 % and a PEV of 45 % (Katersky and Carter 2005) but were similar for greenback flounder which showed a PPV of 17 % and a PEV of 26 % (Carter and Bransden, 2001). Poor digestibility of the diet can lead to reduced efficiency because of poor absorption of nutrients (Bureau et al., 2002). The quality of ingredients can also affect growth efficiency because of an amino acid deficiency which leads to reduced muscle deposition (Carter and Houlihan, 2001). A high inclusion of protein or lipid in fish diets can also reduce growth efficiency caused by catabolism of protein and reduction in growth rates. The use of fish meal and fish oil based diets in this study avoided any concerns regarding digestibility of the diet. The amino acid profile of fish meal is also ideal for growth (NRC, 1993). It is hypothesised that the inaccurate measure of feed intake led to an overestimation of satiation and protein and energy intake. This is supported by the high SGR of 1.6 % found in this study.

The measurement of feed intake is a continuously evolving field and has been discussed extensively by various authors (McCarthy et al., 1993; Jobling et al., 2001b). The precision of measuring feed intake and nutrient uptake in the current experiment was limited due to the following factors. First, counting uneaten pellets is in some instances inaccurate due to striped trumpeter consuming and rejecting,

possibly regurgitating, some pellets which then rapidly disintegrated. Feed ingestion rates reported here could therefore be overestimated, particularly on the satiation ration. Second, striped trumpeter post-larvae did not produce large amount of faeces and stripping the fish would lead to mortalities so traditional measurements of digestibility were not possible.

In comparison with standard feeding rates for striped trumpeter post-larvae culture reared at MRL the optimum ration of 4.0 BM^{-1} was higher. During production, post-larvae and juveniles are fed at a daily ration of 2.5 % to 3.0 % BM d^{-1} with a commercial extruded diet (R. Goldsmid, MRL, University of Tasmania, personal communication). However, the optimum ration found was similar to a recommended ratio of 3.5 % bdw d^{-1} for European sea bass (*Dicentrarchus labrax*), another temperate marine aquaculture species (Eroldogan et al., 2004). A direct comparison of rations and growth and conversion efficiency cannot be made because of other factors which influence conversion efficiency rates such as feed formulation, rearing environment and fish size to name a few (Bureau et al., 2006).

The quality of the experimental diet used was different from the commercial extruded diet used at MRL. The experimental diet was not extruded and was not as water stable as an extruded diet. The experimental diet also incorporated bulking agents such as bentonite and cellulose. It was observed that fish would masticate and then forcefully regurgitate the experimental pellets and would sometime subsequently ingest them again or reject them. The experimental diets yielded similar SGR and intake rates to post-larvae fed with a commercial extruded diet (see Chapter 3) but reduced feed conversion efficiency.

Feed intake of fish is affected by sensory qualities of the feed, pellet size and the quality of the ingredients (Jobling et al., 2001a). The importance of the physical

properties of the feed to match the feeding behaviour of the cultured species should be taken into consideration, for example in Deng et al., (2003) sturgeon were unable to feed on pellets until they were forced to the bottom. Floating pellets may perform better than the sinking pellets used in the current study by prolonging the window for capture. Striped trumpeter post-larvae generally fed at the water surface but were also observed to feed on pellets on the tank bottom. Producing a feed with superior physical and nutritional properties will minimise feed wastage and negative environmental impacts and achieve higher economic gains (Alanara et al., 2001; Read and Fernandes, 2003).

Jaw malformations are typical of fish cultured at MRL and have been linked to walling behaviour (Cobcroft et al., 2001; Cobcroft and Battaglione, 2009). Jaw malformations of varying severity were observed in all of the experimental animals and could have affected the feeding ability of some animals; efforts were made to select only the individuals with functioning mouths. It is also possible that post-larvae were damaged during the course of the experiment because of their flight response to external disturbances and greater interaction with tank surfaces in smaller tanks. To reduce walling tanks were lined with marble backgrounds (Cobcroft and Battaglione, 2009). The feeding activity of experimental animals was not affected by the jaw malformations and the small size of the pellets (1 mm) made them easy to ingest. By the post-larvae stage, striped trumpeter jaws are fully developed and malformations did not increase in severity during the experiment.

In a review by Madrid et al., (2001), rhythms in fish feeding behaviour were found to respond to changing abiotic factors such as light, temperature and oxygen concentration; and also to endogenous responses. In the present study fish were fed continuously throughout the light period managed by an automatic feeding system.

Feeding time and frequency were found to affect nutrient utilisation in cuneate drum (*Nibea miichthioides*) and European sea bass (*Dicentrarchus labrax*) (Bolliet et al., 2001; Wang et al., 2007). However, in a study on Australian snapper (*Pagrus auratus*) by Booth et al., (2008) it was found that the time of feeding did not affect feed utilisation nor did it affect gastric evacuation rates. These studies report opposing findings and it is worth investigating the effects of feeding time, frequency and meal size on striped trumpeter performance on a production scale.

Identifying the optimum ration is useful not only for research but also for its implications on commercial culture. Feeding below the optimum ration has been shown to increase competition and aggressive behaviour in aquacultured finfish species such as sea bream (*Sparus aurata*) (Andrew et al., 2004), Atlantic cod (*Gadus morhua*) (Hatlen et al., 2006), coho salmon (*Oncorhynchus kisutch*) (Ryer and Olla, 1996) and Atlantic salmon (*Salmo salar*) (Noble, et al., 2008). In contrast, flatfish species such as greenback flounder (*Rhombosolea taparina*), yellowtail flounder (*Limanda ferrugine*) and turbot (*Scophthalmus maximus*) do not exhibit aggressive behaviour under restricted rations but still show evidence of inter-individual variation in feed consumption and growth (Carter et al., 1996; Dwyer et al., 2002; Irwin et al., 2002). Striped trumpeter post-larvae did not exhibit physical damage as a result of aggression and the similar coefficient of variation for the three treatment rations indicate an absence of aggressive behaviour even under restricted rations which is desirable for high density culture. Overfeeding, on the other hand, results in increased organic loading. This can cause a decrease in water quality particularly increased ammonia concentrations which can suppress appetite in fish (Ortega et al., 2005). This is particularly important to avoid for striped trumpeter

because striped trumpeter are sensitive to deterioration of water quality (Battaglione and Cobcroft, 2007).

4.5.2 Metamorphosis and development

The protracted post-larval stage of striped trumpeter metamorphosing into juveniles at nine months is highly unique. During this time post-larvae are more susceptible to stress induced mortality than juveniles possibly because of their lower energetic reserves. Adequate energetic reserves are needed to cope with stressors. Studies on rainbow trout and roach (*Rutilus rutilus*), in the wild show that larger individuals with higher lipid reserves survive better under winter starvation conditions (Kirjasniemi and Valtonen, 1997; Biro et al., 2004). Striped trumpeter post-larvae did consume more food compared to juveniles; which agrees with other studies that show a higher feed intake for smaller fish (Jobling, 1994; Fiogbe and Kestemont, 2003). However, this increased intake is offset by the active swimming behaviour of striped trumpeter post-larvae which is a greater energetic burden for small fish (Boisclair and Tang, 2005). This resulted in higher ash and lower total lipid content in post-larvae compared to juveniles and provides an insight into the delicate nature of the post-larvae.

Striped trumpeter juveniles were found to weigh a minimum of 23 g and have at least 4 % lipid in their carcass. Wet weight is a more accurate predictor of metamorphosis than total lipid content because the most marked changes in carcass content are lipid and moisture and is directly correlated with size (Shearer, 1994). Observations of post-larvae and juveniles of similar sizes indicate that a combination of size and nutritional fitness is needed to be fulfilled before metamorphosis is

completed. Using a logistic curve model of wet weight and carcass lipid the point at which 50 % of the population would metamorphose into juveniles was determined as 20 g and 7 % carcass lipid content, all of the post-larvae are predicted to be fully metamorphosed into juveniles when they reach 40 g wet weight and greater than 10 % carcass lipid. In another study by Tracey (2007), 50 % of hatchery reared striped trumpeter post-larvae were predicted to be fully metamorphosed at 150 mm, findings in this experiment are in agreement with the mean length of metamorphosed individuals found to be 150.8 mm. Large post-larvae, which met the minimum size requirement for metamorphosis, were not able to complete metamorphosis due to their inability to meet a nutritional threshold (Shearer, 1994; Thorpe et al., 1998; Berrill et al., 2004).

It should be noted that environmental conditions also have an influence on metamorphosis. Fish under culture conditions experience less diel variability which ensures their consistent growth and reduces stress (Gozlan et al., 1999). Temperature was previously found to have an influence on metamorphosis rates of striped trumpeter post-larvae with fish reared at 12 °C and 14 °C showing higher metamorphosis rates and metamorphosing at smaller sizes compared to fish reared at 16 °C and 18 °C (see Chapter 3). Photoperiod has also been found to have an effect on parr-smolt transformation in Atlantic salmon (Berrill et al., 2003). The effect of photoperiod regimes on striped trumpeter growth is an opportunity for future research.

4.5.3 Conclusion

The success of the striped trumpeter research program has created an impetus to research the rearing of striped trumpeter post-larvae. The striped trumpeter post-larvae is a challenging animal to work with and it is highly desirable to increase its growth rates to hasten metamorphosis into juveniles. Optimising feeding rates will increase efficiency, increase fish performance and promote sustainable aquaculture practices. This study found that a ration of 4.0 % BM d⁻¹ ration of at least 18 % dietary lipid produced high growth rates. Metamorphosis into juveniles should be expected to occur once fish reach a weight of 20 g. Verification of these findings in a commercial setting is also advised to ensure applicability in cage culture.

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Chapter 5

Modelling Nutrient Requirements of Post-larval Striped Trumpeter (*Latris lineata*)

5.1 Abstract

The protein and energy requirements of striped trumpeter (*Latris lineata*) post-larvae were quantified. The study used factorial modelling techniques to estimate protein and energy requirements for maintenance and growth. Metabolic weight exponents were derived from starvation trials on post-larvae and small juveniles held at four temperatures (12, 14, 16 and 18 °C). The relationship between the maintenance requirement for protein and energy and geometric mean wet weight (GMWW) was expressed as $a \times \text{GMWW (kg)}^b$. Metabolic weight exponents of 0.6 and 0.8 for protein and energy respectively were found. The metabolic weight exponent for energy agreed with the generally accepted value of 0.8. The metabolic weight exponent for protein was lower than the generally accepted value of 0.7. Feed, protein and energy intake, wet weight gain, and protein and energy retention efficiencies were collected from previously conducted growth trials on striped trumpeter post-larvae during metamorphosis into juveniles. These studies investigated the optimum rearing temperature and the effect of feeding fish at different ration levels with diets of different dietary lipid content. Chemical composition of fish was measured using a comparative slaughter technique. Digestibility could not be estimated because of difficulties in handling striped trumpeter post-larvae and the small volume of faeces that the biomass produced. Estimates were expressed on an absolute feed intake basis. Linear regression analysis was used to determine protein and energy requirements for maintenance and maximum growth using metabolic weight exponents of 0.7 for protein and 0.8 for energy. To maintain wet weight a requirement of $6.99 \text{ g kg}^{-0.8} \text{ d}^{-1}$ of feed, a protein intake requirement of $1.84 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and an energy intake requirement of 116.06 kJ

$\text{kg}^{-0.8} \text{d}^{-1}$ were found. Requirements to achieve maximum growth were $34.87 \text{ g kg}^{-0.8} \text{d}^{-1}$ of feed, a protein intake of $14.26 \text{ g kg}^{-0.7} \text{d}^{-1}$ and an energy intake of $653.53 \text{ kJ kg}^{-0.8} \text{d}^{-1}$, this translated to a ratio of $21.82 \text{ g protein MJ}^{-1} \text{ energy}$. This is the first study to investigate protein and energy requirements for maintenance and growth of striped trumpeter post-larvae during their metamorphosis into juveniles and provides valuable data for future diet formulation and nutrition research.

5.2 Introduction

An increasingly adopted approach to determining nutrient requirements in fish is factorial modelling which quantifies requirements based on determining relationships between nutrient intake and nutrient retention (Lupatsch et al., 1998; Hauler and Carter, 2001; Carter et al., 2008). Factorial models have been used to determine protein and energy requirements for commercially important aquaculture species such as sea bream (*Sparus aurata*) (Lupatsch et al., 1998), European sea bass (*Dicentrarchus labrax*) (Lupatsch et al., 2001), white grouper (*Epinephelus aeneus*) (Lupatsch and Kissil, 2005), barramundi (*Lates calcarifer*) (Glencross, 2008), and mullet (*Argyrosomus japonicus*) (Pirozzi et al., 2008). A key component of a factorial model is the use of a metabolic weight to reflect the allometric pattern of growth in fishes. Using data collected from starved fish, a weight exponent for energy of 0.8 has been consistently found across several fish species (Brett and Groves, 1979; Lupatsch et al., 1998; Lupatsch et al., 2003). A weight exponent for protein varies but a value of 0.7 is generally accepted (Lupatsch et al., 2003; Carter et al., 2008). It has also been found that models of protein and energy loss used to determine metabolic weight exponents are unaffected by water temperature (Lupatsch et al., 2001; Lupatsch et al., 2003; Lupatsch and Kissil, 2005; Pirozzi et al., 2008).

The striped trumpeter (*Latris lineata*) is being researched as a potential alternative fish species for the Tasmanian sea cage aquaculture industry. Previous research has made significant contributions to the understanding of the broodstock management and larviculture of the striped trumpeter (Battaglione and Cobcroft, 2007). The development of weaning strategies has made the production of striped trumpeter post-larvae more reliable and cost-effective (see Chapter 2). Culture of

post-larvae is complicated by its prolonged duration (see Chapter 1 and 7; Battaglione and Cobcroft, 2007; Tracey 2007). Some of the requirements for successful post-larvae culture have been investigated including the optimum rearing temperature and feeding regimes (see Chapter 3 and Chapter 4).

In the current chapter, starvation trials with striped trumpeter post-larvae were conducted to derive metabolic weight exponents for protein and energy and were applied to data from previous growth trials reported in Chapter 3 and Chapter 4 to develop factorial models to predict protein and energy requirements for maintenance and maximum growth. Nutrient intake is expressed in terms of absolute intake and not on a digestible basis because its accurate determination was not possible for post-larvae. The study aimed to further explore the growth patterns of striped trumpeter post-larvae and to establish a foundation for future nutrition research on striped trumpeter.

5.3 Materials and methods

5.3.1 *Modelling protein and energy requirements: determination of weight exponents*

Loss of whole body energy and protein during 10 days without food was used to estimate the weight exponents for modelling protein and energy requirements (Lupatsch et al., 1998, Lupatsch et al., 2003). Striped trumpeter post-larvae of 1, 4, 8, 16 and 32 g and juveniles of 120 g in wet weight were sampled from production runs at the Marine Research Laboratories, Tasmania (MRL). Twenty fish of 1 g were held in each replicate tank ($n = 4$) while a single fish was held in each replicate tank for the other weight categories ($n = 8$). Fish were randomly stocked and held in 150 L

tanks with marbled sides for 10 d without food at four different temperatures (12, 14, 16 and 18 °C). During the starvation trials, other culture parameters were maintained at optimum levels pH, 8.0 ± 0.1 , salinity, 33.9 ± 0.2 ppt, oxygen saturation, 98.9 ± 0.9 %. The Animal Ethics Committee of the University of Tasmania deemed it prudent to restrict the starvation period to 10 d due to this being the first study to withhold food from striped trumpeter post-larvae (Approval number A0008719). A comparative slaughter technique was used to determine the energy and protein lost during starvation (Lupatsch et al., 1998, 2001; Bureau et al., 2002). Metabolic weight exponents for protein and energy were determined by fitting a power function to the nutrient loss and geometric mean wet weight data.

Fish were held in the same tanks described in Chapters 3 and Chapter 4. Each row of tanks was equipped with a heat exchanger to maintain the desired water temperature. Lighting was provided by fluorescent globes emitting $2.80 \mu\text{mol} \pm 0.09$ (mean \pm SE) of light, measured from the water surface at the middle of each tank. Photoperiod was maintained at 16-h light:8-h dark throughout the experiment. Water supply to the tanks was filtered through 1 micron bag filters. Flow rates to the tanks were maintained at 162 L h^{-1} . For initial chemical composition of fish from each weight class, fish were taken from the production tanks and starved for 24 h then euthanased using an overdose of Aqui-S™ (Aqui-S, New Zealand) (5 ml in 20 L seawater). Five replicates of 20 fish were taken for the initial composition of 1 g fish while ten individual fish were taken as an initial sample for trials with fish 4, 8, 16, 32 and 120 g in weight. All of the fish were measured individually for wet weight and total length before the starvation period. At the end of the ten day starvation period, fish were again measured for wet weight and total length and euthanased

with an overdose of Aqui-S™. Samples were stored at -20 °C prior to chemical analysis.

5.3.2 Chemical analysis

Samples were autoclaved and freeze dried to a constant weight prior to whole-body composition analysis (Williams et al., 1995). The dry weights of individual samples were determined by freeze drying to a constant weight. Freeze dried samples of the diet and individual fish were ground to a homogenous powder prior to chemical analysis. Crude protein was determined using an elemental analyser (Thermo Finnigan 1112 Series elemental analyser, N X 6.25); total lipid was determined using Bligh and Dyer (1959); and ash was determined by combustion at 550 °C for 16 h (AOAC, 1995). Energy content was calculated using conversion factors for protein (23.6 kJ g⁻¹) and lipids (36.2 kJ g⁻¹) (Brafield, 1985). Three replicate measurements for crude protein, total lipid and two replicate measurements for ash were performed for each sample.

5.3.3 Calculations

The initial protein (g) and energy (kJ) content of the starved fish was estimated using the mean chemical composition of the fish taken as initial samples. Loss of protein (g) and energy (kJ) were calculated by subtracting final composition from the calculated initial composition. Loss was expressed on a daily basis.

5.3.4 Factorial modelling

The metabolic weight exponents were derived from log-log plots of daily protein and energy loss of each replicate sample and the geometric mean wet weight during the starvation period. Geometric mean wet weight was calculated as $(\text{initial wet weight} \times \text{final wet weight})^{0.5}$ (Lupatsch et al., 1998). A power function was fitted to determine the relationship between wet weight and loss of protein and energy per day. Each temperature was plotted independently along with an overall plot for loss of protein and energy.

The metabolic weight exponents for protein and energy were applied to growth data from previous growth trials (see Chapter 3 and Chapter 4). The experimental design and conditions of these trials are detailed in their respective chapters. Linear regression was used to calculate the maintenance requirements of wet weight, whole body crude protein and whole body energy content and expressed as dry material, protein and energy intake. Statistical significance was accepted at $p = 0.05$. Statistical analyses were performed using SPSS version 15.0 statistical software.

5.4. Results

5.4.1 Determination of the metabolic weight exponents

Mortalities were observed during the starvation period, particularly for the smaller weight classes. The lowest survival in the pooled tanks for the post-larvae of 1g in wet weight was 75 % of an initial 20 fish per tank. The lowest survival in the

larger sizes of post-larvae (4 g to 120 g) was 62.5 % of an initial eight individual fish per temperature. The daily loss of protein (g) and energy (kJ) was calculated for each weight class of striped trumpeter (1, 4, 8, 16, 32 and 120 g) and plotted against the geometric mean (initial weight and starved weight after 10 days) and fitted using a power function to reflect an allometric relationship (Fig. 5.1 and Fig. 5.2). An overall model for protein and energy loss at the four temperatures is presented (Fig. 5.3). Equations for the power function showing the relationship between protein loss ($\text{g d}^{-1} \text{ fish}^{-1}$) and geometric mean wet weight (kg) at each temperature and the overall model are summarised in Table 5.1.

Table 5.1: Equations for power functions showing the metabolic weight exponent (b) for protein for striped trumpeter post-larvae (1, 4, 8, 16, 32 and 120 g wet weight) starved for 10 d. Protein loss ($\text{g d}^{-1} \text{ fish}^{-1}$) = $a \times \text{Geometric mean WW (kg)}^b$

Temperature	a	b	r^2	n	F	p
12 °C	0.45	0.55	0.948	6	72.4	0.001
14 °C	0.34	0.52	0.925	6	49.7	0.002
16 °C	0.42	0.53	0.940	6	63.1	0.001
18 °C	0.98	0.66	0.843	6	21.5	0.010
Overall (12-18 °C)	0.50	0.57	0.875	24	154.3	<0.001

Equations for the power function showing the relationship between energy loss ($\text{kJ d}^{-1} \text{ fish}^{-1}$) and geometric mean wet weight (kg) at each temperature and the overall model are summarised in Table 5.2.

Table 5.2 Equations for power functions showing the metabolic weight exponent (b) for energy for striped trumpeter post-larvae (1, 4, 8, 16, 32 and 120 g wet weight) starved for 10 d. Energy loss ($\text{kJ d}^{-1} \text{ fish}^{-1}$) = a Geometric mean WW (kg) ^{b}

Temperature	a	b	r^2	n	F	p
12 °C	79.47	0.81	0.903	6	37.4	.004
14 °C	56.09	0.76	0.905	6	37.9	.004
16 °C	66.47	0.77	0.912	6	41.6	.003
18 °C	76.33	0.76	0.929	6	52.7	.002
Overall (12-18 °C)	68.65	0.77	0.905	24	209.7	<0.001

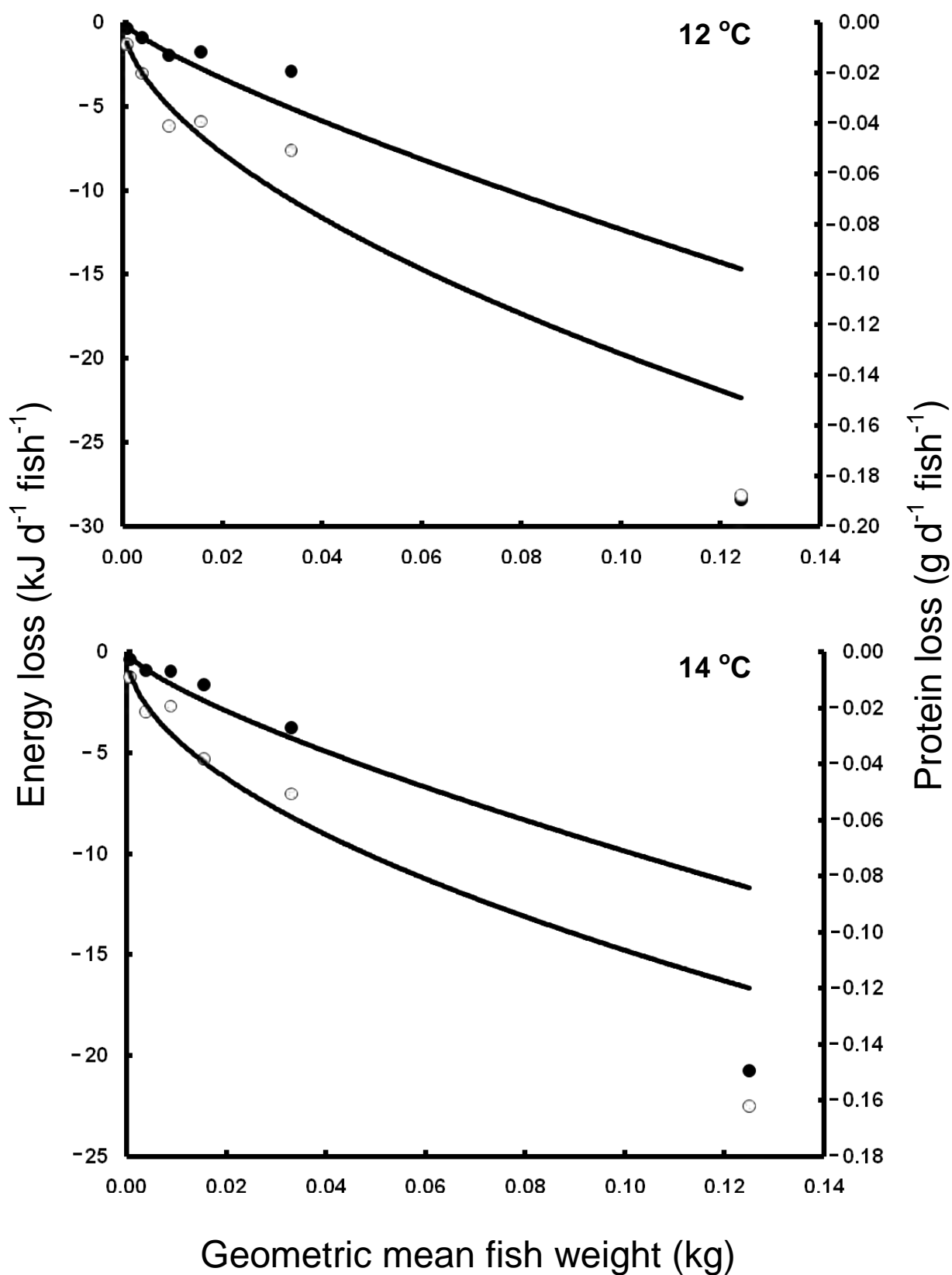


Figure 5.1: Protein ($\text{g d}^{-1} \text{ fish}^{-1}$) and energy ($\text{kJ d}^{-1} \text{ fish}^{-1}$) loss in striped trumpeter after a starvation of 10 days at 12 and 14°C. Protein loss is represented by (O) and energy loss is represented by (●).

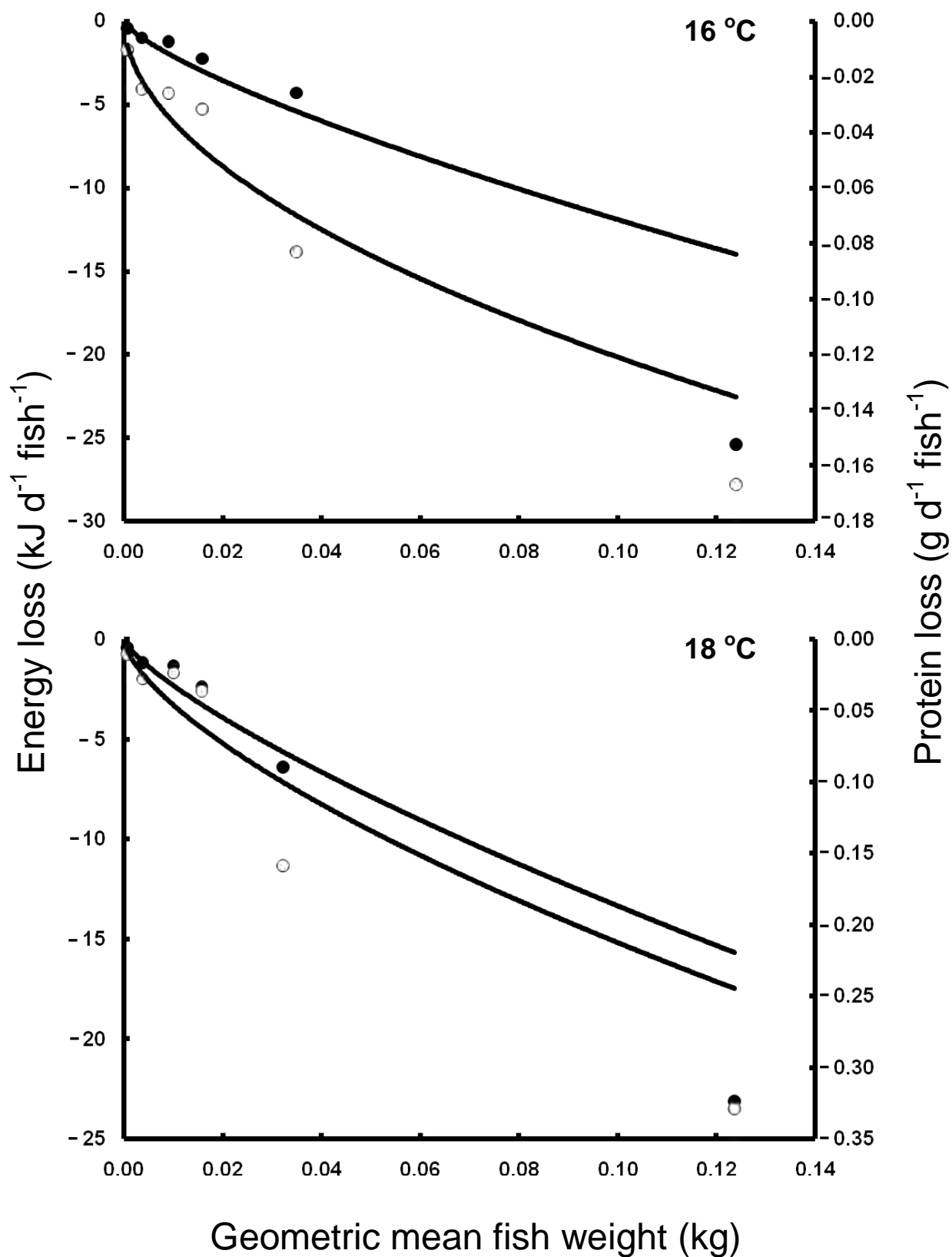


Figure 5.2: Protein ($\text{g d}^{-1} \text{ fish}^{-1}$) and energy ($\text{kJ d}^{-1} \text{ fish}^{-1}$) loss in striped trumpeter after a starvation of 10 days at 16 and 18°C. Protein loss is represented by (○) and energy loss is represented by (●).

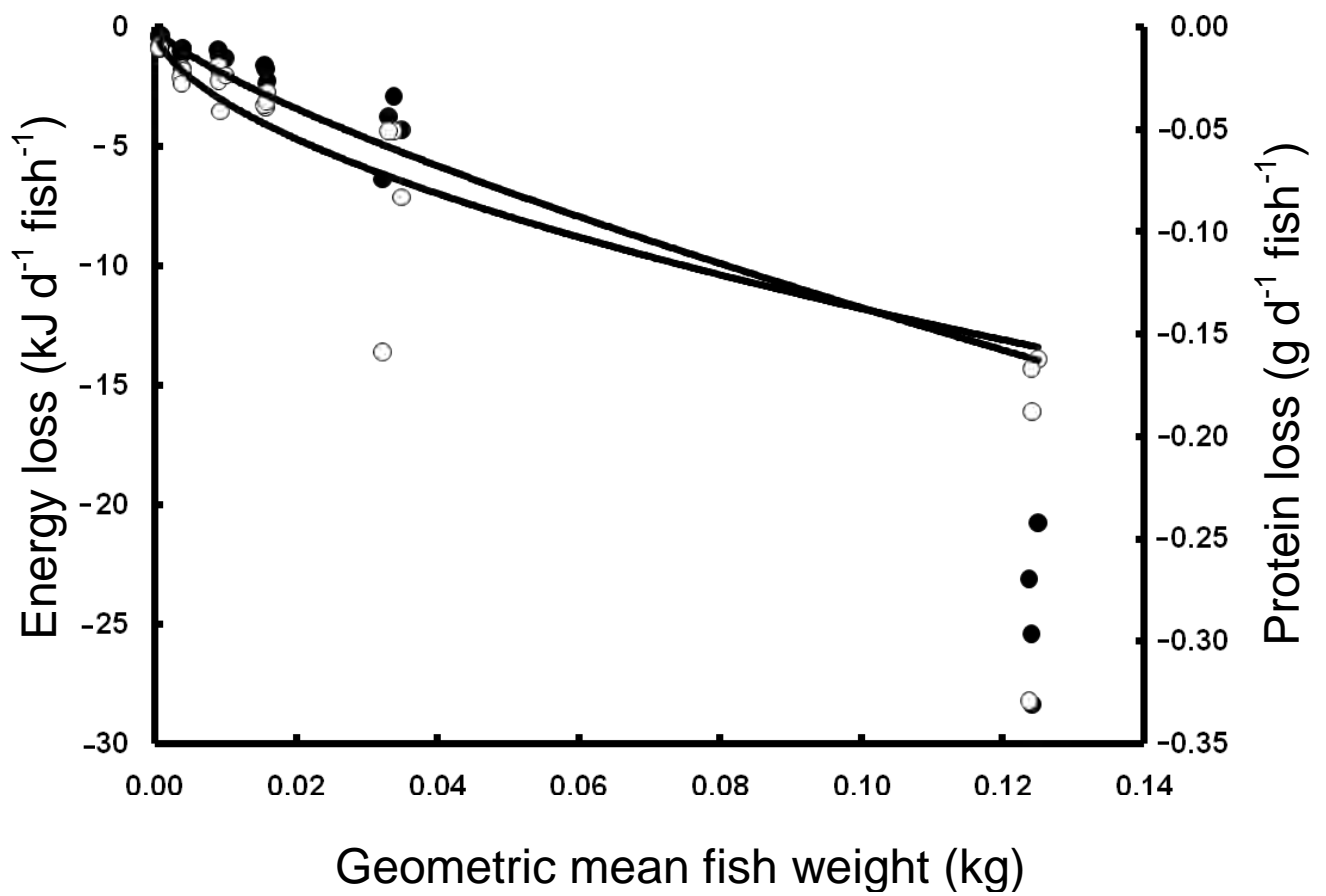


Figure 5.3: Overall model of protein (g d⁻¹ fish⁻¹) and energy (kJ d⁻¹ fish⁻¹) loss in striped trumpeter after a starvation of 10 days. Protein loss is represented by (○) and energy loss is represented by (●).

5.4.2 Factorial models

Significant linear regressions were found between weight gain and feed intake (Fig. 5.4), protein intake (Figs. 5.5) and energy intake (Fig. 5.6). The linear regression equations are summarised in Table 5.3. The feed input required to maintain wet weight was predicted to be $6.99 \text{ g kg}^{-0.8} \text{ d}^{-1}$. Two metabolic weight exponents for protein were used, 0.6 derived from the overall protein loss model and 0.7 which is the generally accepted metabolic weight exponent for protein (see section 5.2 Introduction). Protein intake required to maintain wet weight was predicted to be $1.84 \text{ g kg}^{-0.7} \text{ d}^{-1}$ using a metabolic weight exponent of 0.7 or $1.21 \text{ g kg}^{-0.6} \text{ d}^{-1}$ using a metabolic weight exponent of 0.6. The energy intake required to maintain wet weight was predicted to be $116.06 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$.

Table 5.3: Equations of linear regressions found between weight gain and feed intake, protein intake and energy intake ($n = 44$). The * indicates the appropriate metabolic weight exponents were used for each equation. $\text{Intake (g kg}^* \text{ d}^{-1}) = a \text{ Weight Gain (g kg}^* \text{ d}^{-1}) + b$

Intake (g kg* d ⁻¹)	Metabolic Weight Exponent	r^2	F	p	a	b
Feed intake	0.8	0.928	538.88	<0.001	1.46	6.99
Protein intake	0.7	0.938	632.31	<0.001	0.65	1.84
Protein intake	0.6	0.936	610.24	<0.001	0.64	1.21
Energy intake	0.8	0.938	635.68	<0.001	28.14	116.06

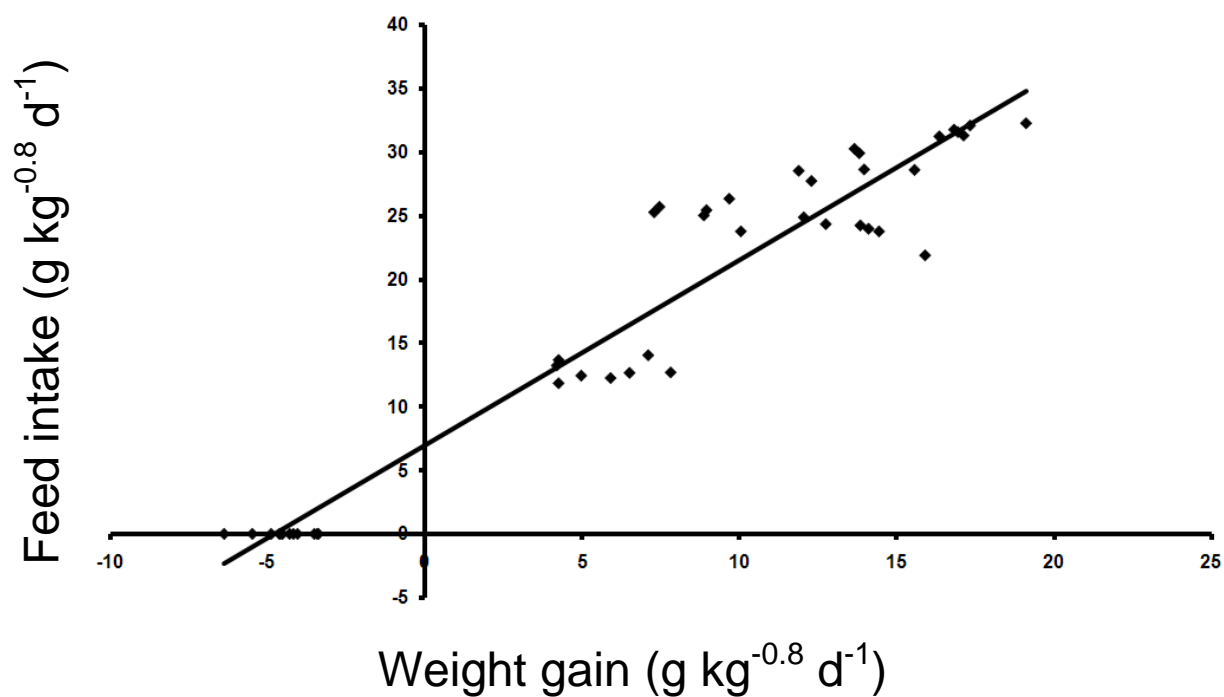


Figure 5.4: Linear regression model for the relationship between and feed intake (g kg^{-0.8} d⁻¹) and weight gain (g kg^{-0.8} d⁻¹) for striped trumpeter post-larvae and juveniles (8 g to 50 g) reared at 12, 14, 15, 16 and 18 °C.

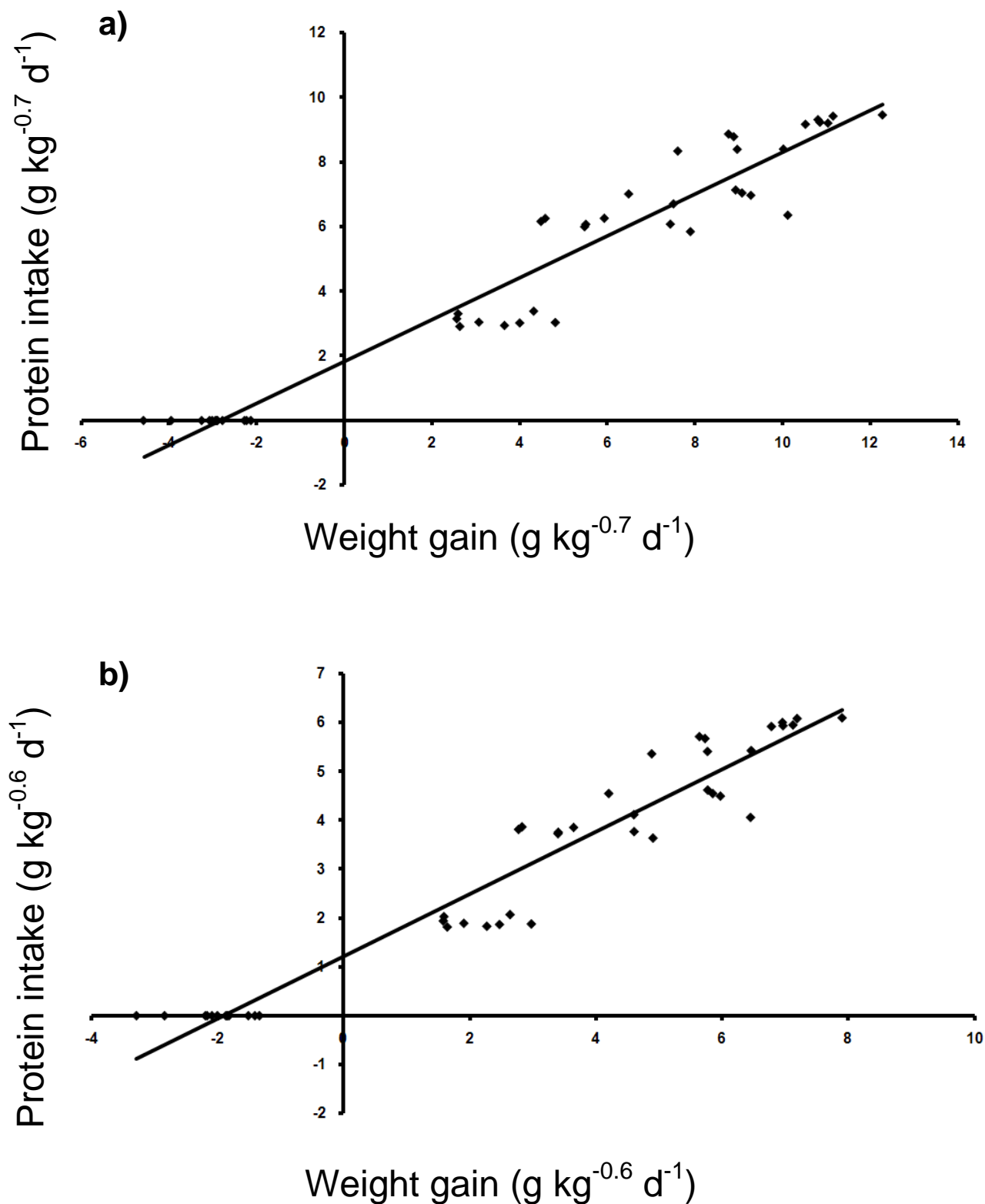


Figure 5.5: Linear regression models for the relationship between protein intake ($\text{g kg}^{-0.7 \text{ or } -0.6} \text{ d}^{-1}$) and weight gain ($\text{g kg}^{-0.7 \text{ or } -0.6} \text{ d}^{-1}$); metabolic weight exponents of (a) 0.7 and (b) 0.6 were applied to the growth of striped trumpeter post-larvae and juveniles (8 g to 50 g) reared at 12, 14, 15, 16 and 18 °C.

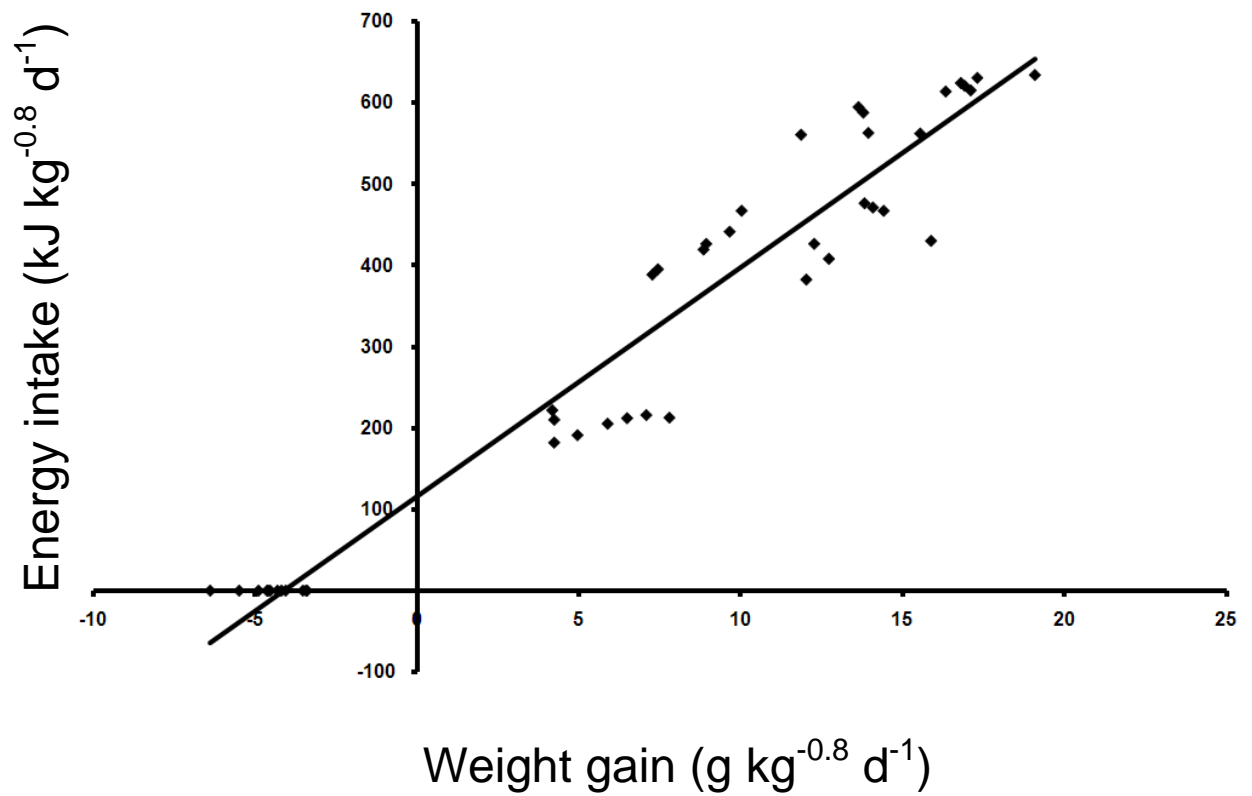


Figure 5.6: Linear regression model for the relationship between energy intake (kJ kg^{-0.8} d⁻¹) and weight gain (g kg^{-0.8} d⁻¹) for striped trumpeter post-larvae and juveniles (8 g to 50 g) reared at 12, 14, 15, 16 and 18 °C.

Significant linear regressions were found between protein retention and protein intake (Fig. 5.7) and between energy retention and energy intake (Table 5.4; Fig. 5.8). The equations of these linear regressions are summarised in Table 5.4. The protein intake requirement for maintenance of whole body protein was $2.18 \text{ g kg}^{-0.7} \text{ d}^{-1}$ using a metabolic weight exponent of 0.7, and $1.67 \text{ g kg}^{-0.6} \text{ d}^{-1}$ using a metabolic weight exponent of 0.6. The energy intake requirement for maintenance of whole body energy was $184.02 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$. The slope coefficients represent a cost of production (Carter et al., 2008) and equated to 3.44 and 3.37 for one unit of protein using metabolic weight exponents of 0.7 and 0.6, respectively. The cost of production for one unit of energy was 2.82. The reciprocal of these values describes the efficiency of utilisation for growth above maintenance levels (Carter et al., 2008) and the values for protein are 29.1 % and 30.2 % using metabolic weight exponents of 0.7 and 0.6, respectively. The efficiency of utilisation for energy was 35.5 %.

Table 5.4: Equations of linear regressions found between weight gain and feed intake, protein intake and energy intake ($n = 44$). The * indicates appropriate metabolic weight exponents were used for each equation. Intake ($\text{g kg}^* \text{ or } \text{kJ}^{-0.8} \text{ d}^{-1}$) = a Retention ($\text{g kg}^* \text{ or } \text{kJ}^{-0.8} \text{ d}^{-1}$) + b

Nutrient	Metabolic weight exponent	r^2	F	p	a	b
Protein	0.7	0.938	639.08	<0.001	3.44	2.18
Protein	0.6	0.927	533.98	<0.001	3.37	1.67
Protein	0.8	0.940	663.87	<0.001	2.82	184.02

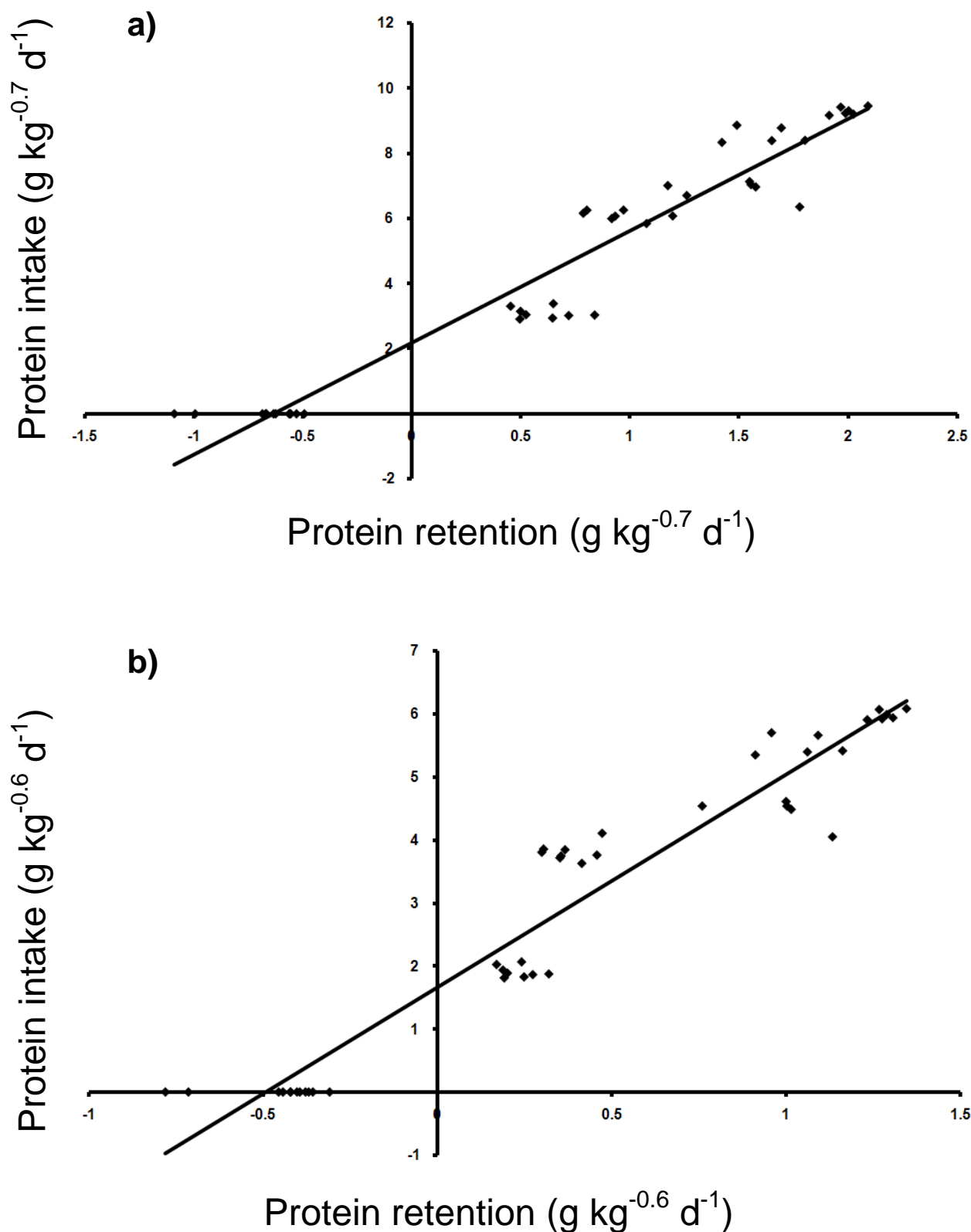


Figure 5.7: Linear regression models for the relationship between protein intake ($\text{g kg}^{-0.7}$ or $\text{g kg}^{-0.6} \text{ d}^{-1}$) and protein retention ($\text{g kg}^{-0.7}$ or $\text{g kg}^{-0.6} \text{ d}^{-1}$) with metabolic weight exponents of (a) 0.7 and (b) 0.6 for striped trumpeter post-larvae and juveniles (8 g to 50 g) reared at 12, 14, 15, 16 and 18 °C.

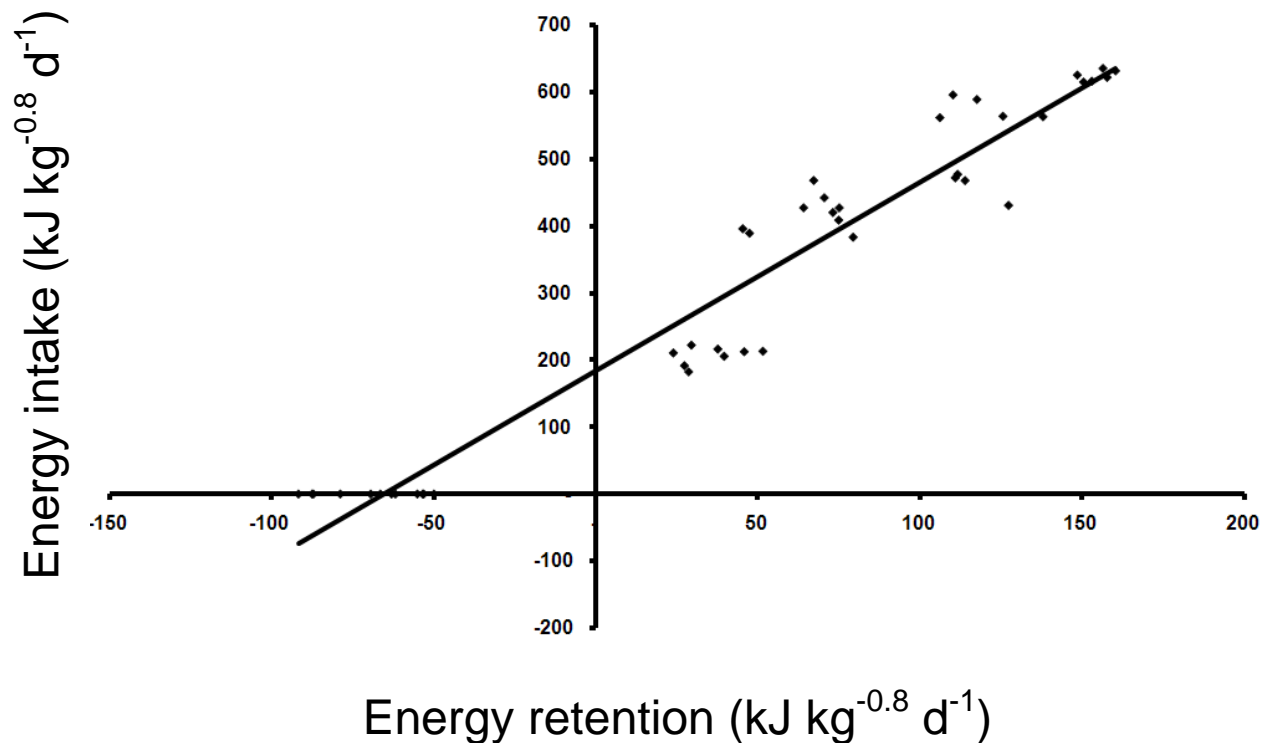


Figure 5.8: Linear regression model for the relationship between energy intake ($\text{kJ kg}^{-0.8} \text{ d}^{-1}$) and energy retention ($\text{kJ kg}^{-0.8} \text{ d}^{-1}$) and for striped trumpeter post-larvae and juveniles (8 g to 50 g) reared at 12, 14, 15, 16 and 18 °C.

The maximum weight gain was $19.10 \text{ g kg}^{-0.8} \text{ d}^{-1}$ (Fig. 5.4) requiring a feed intake of $34.87 \text{ g kg}^{-0.8} \text{ d}^{-1}$. Protein intake required to achieve the maximum wet weight gain are $14.26 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $13.43 \text{ g kg}^{-0.6} \text{ d}^{-1}$ for the two metabolic weight exponents at an energy intake of $653.53 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$ (Table 5.3). The optimum dietary protein to dietary energy for maximum weight gain was approximately $21.82 \text{ g protein MJ}^{-1} \text{ energy}$. Maximum protein retention was $2.09 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $1.35 \text{ g kg}^{-0.6} \text{ d}^{-1}$ for the two metabolic weight exponents for protein. Maximum energy retention was $160.22 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$. A protein intake of $9.37 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $6.22 \text{ g kg}^{-0.6} \text{ d}^{-1}$ were required to achieve the maximum protein retention rates. An energy intake of $635.84 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$ was required to meet the maximum energy gain (Table 5.2).

5.5 Discussion

This is the first study to use factorial modelling techniques to estimate nutrient requirements and nutrient retention efficiencies of striped trumpeter post-larvae during their metamorphosis into juveniles. This growth stanza is of scientific and commercial interest because of the prolonged duration of the post-larvae life history stage and the susceptibility of the post-larvae to stressors (Battaglione and Brown, 2006; Battaglione and Cobcroft, 2007).

Metabolic weight exponents for protein and energy were derived from the loss of these nutrients (Lupatsch et al., 1998; Lupatsch et al., 2003; Lupatsch and Kissil; 2005). Individual fish were starved because of the difficulty in tagging the fish. Post-larval striped trumpeter are particularly delicate and tagging to mark individuals in a pooled group would have caused significant trauma and injury to the

animals and would likely have distorted the nutrient depletion data. Starving individual fish was considered the best compromise and the results suggest the fish were not severely stressed by isolation. As in other studies the value of the metabolic weight exponent for energy loss did not appear to be affected by temperature (Pirozzi et al., 2008). An overall metabolic weight exponent of 0.77 was found which was close to the generally accepted metabolic exponent for energy of 0.80 used in other fish factorial models (Brett and Groves, 1979; Lupatsch et al., 2003; Carter et al., 2008). The highest metabolic weight exponent for protein was found in fish starved at 18 °C (0.67), followed by those starved at 12 °C (0.58). Fish starved at 14 °C (0.52) and 16 °C (0.54) showed similar metabolic weight exponents for protein. An overall metabolic weight exponent of 0.6 for protein was found. Results from the study investigating optimum temperatures (see Chapter 3) confirm these results. Fish reared at 18 °C showed reduced growth rates and delayed development.

The relatively short starvation period of ten days was probably not long enough to obtain a consistent loss of reserves across very different fish weights. This may have lead to errors in calculating changes in chemical composition based on the composition of initial fish (Brafield, 1985). The Animal Ethics Committee decided to err on the side of caution and recommended the relatively conservative starvation period. Unfortunately 64 g fish were not available when the starvation trials were conducted and this meant a gap in the progression of sizes measured. Although the models had high r^2 values the power function could have provided more accurate estimates of nutrient losses with a sample of fish weighing 64 g partly because of the spread of weights. The predicted relationships underestimated the losses in larger fish. Large fish would be predicted to lose protein and energy reserves more slowly than smaller fish (Jobling, 1994). One reason for the observed higher losses in larger

fish may have been the disproportionately increased protein and energy expenditure caused by isolation stress (Pickering et al., 1987; Allen et al., 2009). It is recommended that future attempts to refine this preliminary factorial model adopt longer starvation periods and attempt to assess depletion of reserves of fish held in groups to arrive at more accurate metabolic weight exponents.

Digestibility data could not be collected for striped trumpeter post-larvae because of the inadequate volume of faeces produced by the animals and the inability of the post-larvae to tolerate physical stripping. Digestibility might be expected to be high because the growth data from Chapter 3 and Chapter 4 were obtained from fish fed with a fish meal and fish oil based diet which should be highly digestible and possess the appropriate amino acid and fatty acid profiles to support fish growth (NRC, 1993). However the relatively low retention efficiencies indicate some nutrient losses so lower than expected digestibility cannot be discounted (see below). Results from Chapter 3 and Chapter 4 were used to provide growth data for the factorial models. Chapter 3 shows data from post-larvae fed to satiation reared at 12, 14, 16 and 18 °C and Chapter 4 shows data from restricted feeding for post-larvae reared at 15 °C. The 100 % ration treatment from Chapter 4 was not included in the factorial models because overfeeding underestimated nutrient retention (see Chapter 4).

The current research predicted that the protein and energy requirement for maintenance of wet weight were $1.84 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $116.06 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$, respectively. Maintenance requirements for whole body crude protein and energy content were $2.18 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $184.02 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$ based on the linear regressions for protein and energy retention. These results reflect an early stage of diet formulation for striped trumpeter post-larvae and small juveniles. Maintenance

requirements for wet weight were lower than those required to maintain whole body crude protein and energy. Maintenance requirements for whole body crude protein and energy provide a more useful indication of nutrient requirements because it is independent of the chemical composition of wet weight gain which can be influenced by endogenous and exogenous factors (Shearer, 1994). This has implications for metamorphosis of post-larvae into juveniles because a chemical composition threshold is required to be met before metamorphosis is completed (see Chapter 4 and Chapter 6). Maintenance requirements found in this study were similar to values found for Atlantic salmon which were $2.19 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $184.77 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$ (Carter et al., 2008), however the study on Atlantic salmon was conducted at 19°C which is beyond the optimum temperature for salmon. A study of three temperate species by Lupatsch, et al., (2003) showed that digestible energy intake for maintenance of 42.52, 40.14 and $30.81 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$ were lower for sea bream, sea bass and white grouper (*Epinephelus aeneus*), respectively. Further, Bureau et al., (2002) reports that a maintenance requirement of 40-60 kJ metabolisable energy body weight $^{-0.8} \text{ day}^{-1}$ is generally applicable for fish species reared at optimum temperature.

The efficiency of protein utilisation was 29 % and was much lower compared to those for gilthead sea bream, 53 %; European sea bass, 53 % and white grouper 56 % (Lupatsch et al., 2003) and 53 % for Atlantic salmon (Carter et al., 2008). The efficiency of energy retention was 40 % and was also lower than those found for gilthead sea bream, 67 %; European sea bass 69 % and white grouper 69 % (Lupatsch et al., 2003) and 67 % for Atlantic salmon (Carter et al., 2008). The differences in nutrient requirements and lower nutrient utilisation efficiency of striped trumpeter post-larvae can be attributed to several factors. Firstly, the results

are not presented on a digestible basis due to difficulties mentioned earlier. Nutrient utilisation measurements would be improved by measuring digestibility of ingredients. Secondly, the lower retention rates could be caused by the overestimation of feed intake. Although care was taken to measure as much of the feed ingested as possible, it is possible that feed intake measurements were inaccurate (see Chapter 3 and Chapter 4). Thirdly, optimum diet formulation for striped trumpeter post-larvae is not known and the diets used for both growth trials may not have been optimised to meet the requirements of striped trumpeter post-larvae. This was mitigated by using high quality fish meal and fish oil in the diets and using an inclusion rate of > 40 % for dietary protein based on diets used for commercial production (see Chapter 6). Fourthly, the culture conditions, particularly the tank surface area and volume, might have contributed to lower retention rates by limiting the available space for the post-larvae to exercise. It has been found that fish that are allowed to exercise have higher growth rates and improved food conversion by increasing the aerobic potential of red and white muscle (Davison, 1997). Other possible causes for the low retention are the impairment of feeding by jaw malformations and the highly active pelagic nature of the post-larvae.

The maximum growth rate of striped trumpeter post-larvae was $19.10 \text{ g kg}^{-0.8} \text{ d}^{-1}$, maximum protein gain was $2.09 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and maximum energy gain was $160.22 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$. The feed intake required to achieve the maximum growth was $34.87 \text{ g kg}^{-0.8} \text{ d}^{-1}$. The protein intake and energy intake required to achieve the maximum growth in wet weight were $14.26 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $653.53 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$, respectively. Nutrient requirements for maximum protein and energy gain were $9.37 \text{ g kg}^{-0.7} \text{ d}^{-1}$ and $635.84 \text{ kJ kg}^{-0.8} \text{ d}^{-1}$, respectively. This predicted an optimal protein to energy ratio of $21.82 \text{ g protein MJ energy}^{-1}$ was found for maximum growth in wet

weight. These estimates are useful in diet formulation for striped trumpeter post-larvae to optimise the retention of protein by meeting energetic demands using lipid without overfeeding with lipid leading to depressed growth due to reaching satiation before an adequate protein amount is ingested (Halver and Hardy, 2002).

In conclusion, the research in the current chapter presents the maintenance requirements and nutrient retention efficiencies for protein and energy for striped trumpeter post-larvae for the entire size range during a prolonged life-history stage. This is an important stage because post-larvae are delicate animals and it is desirable to optimise culture parameters to promote metamorphosis into juveniles as early as possible. Intake is expressed on an absolute basis because of difficulties encountered in measuring digestibility. This study can be refined in the future by measuring digestibility and prolonging the starvation period. Further empirical testing of findings presented in this study will lead to optimum diet formulations for striped trumpeter post-larvae and small juveniles.

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Chapter 6

Chemical Composition of Striped Trumpeter (*Latris lineata*) Throughout Its Life Cycle

6.1 Abstract

The chemical, mineral and elemental composition of striped trumpeter (*Latris lineata*) was studied over its life cycle by collecting samples from production runs reared under current “best practice” conditions. Specimens collected reflect composition and retention under known optimal conditions. Allometric relationships were found between total lipid content and moisture content and wet weight (g) (WW) ($a \times WW^b$). Total lipid content (% WW) increased with increasing WW ($4.7 \times WW^{0.2}$), while the inverse was observed with moisture content (% WW) ($76.4 \times WW^{-0.3}$). Energy content (kJ g⁻¹) was also allometrically related to WW ($5.5 \times WW^{1.0}$). Crude protein content (17.8 ± 0.1 % WW) and ash content (4.5 ± 0.2 % WW) did not vary significantly. Growth of post-larvae was also observed to be different from growth of juvenile striped trumpeter. The chemical, mineral and elemental composition of striped trumpeter (*Latris lineata*) was studied over its life cycle by collecting samples from production runs reared under current “best practice” conditions. Specimens collected reflect composition and retention under known optimal conditions. The data presented in this study can find application in nutrient requirement and diet formulation studies where knowledge of accretion is needed. This is the first study to investigate composition of striped trumpeter throughout its life-cycle and is also one of the few studies of this scope conducted on fish species. Results provide a useful baseline for reference for comparison with other commercially cultured species and future production runs and growth trials on striped trumpeter.

6.2 Introduction

The striped trumpeter (*Latris lineata*) is a temperate marine finfish found in Tasmanian waters that has been selected as a candidate species for aquaculture (Battaglione and Cobcroft, 2007). Culture of this species is complicated by its unusual prolonged neustonic “paperfish” or post-larval life history stage which can last up to nine months (Furlani and Ruwald, 1999; Tracey, 2007). Nutrient requirements for striped trumpeter larvae have been identified resulting in established hatchery protocols for producing larvae (Bransden et al., 2005a,b; Battaglione and Cobcroft, 2007). Research is now directed toward collecting empirical data on rearing of post-larvae and improving growth rates with the goal of hastening metamorphosis into juveniles and consequently increasing survival. The production of striped trumpeter juveniles for a sea cage trial provided an opportunity to collect baseline data on chemical, mineral and trace element composition over the life cycle of a species with an unusual life history stage and at the start of aquaculture development.

This is the first study on chemical composition of striped trumpeter throughout its complete life cycle and establishes a baseline reference for comparison of chemical composition. Baseline data chemical composition provides an initial step towards to understanding nutrient requirements. For example, composition data can be used to determine nutrient requirements by comparing composition of eggs and larvae or by comparing nutrient loss and conservation during feeding and starvation (Sargent, 1995; Izquierdo and Fernandez-Palacios, 1997). Chemical composition studies for striped trumpeter have previously been

conducted on eggs from cultured and wild sources (Morehead et al., 2001) and larvae (Brown et al., 2005) and broodstock (Bransden et al., 2007).

Life-time or long-term studies of the crude chemical composition (crude protein, total lipid, moisture, and ash) of cultured fish are few and the most complete is on Atlantic salmon (*Salmo salar*) by Shearer et al., (1994). Other useful examples on marine species include the sea bream (*Sparus aurata*) (Lupatsch et al., 1998), and European sea bass (*Dicentrarchus labrax*) (Lupatsch et al., 2001). Findings from these studies agree that moisture and total lipid are inversely related whereas crude protein and ash content remain constant throughout the life cycle. Endogenous (e.g. life history stage, sexual maturity) and exogenous factors (e.g. temperature, salinity, and exercise) can influence the total lipid reserves of fish and consequently the energy content (Shearer, 1994). Crude protein content does not vary greatly and fish conserve crude protein, there is a preference to store and then utilise non-protein energy in the form of lipids more rapidly (Carter and Brafield, 1991, Bureau et al., 2002; Sargent et al., 2002).

Inorganic elements are also required for the maintenance of fish growth and health (Watanabe et al., 1997; Lall, 2002). Minerals and trace elements are essential and have many important roles such as in specific metabolic pathways, skeletogenesis, and maintenance of colloidal systems and as components of enzymes (Lall, 2002). The following are classed as essential minerals: calcium, phosphorus, sodium, potassium, and the following as essential trace elements: zinc, manganese, copper, iron, chromium, cobalt, fluorine, iodine, molybdenum, and selenium (NRC, 1993; Lall, 2002). A mineral deficiency will firstly result in less overt signs such as decreased growth and lower feed utilisation efficiency (Roy and Lall, 2003) and are often manifested through skeletal deformities (Lall and Lewis-McCrea, 2007). The

study of requirements for minerals and trace elements is made difficult by the ability of fish to absorb some of these elements from the aquatic environment. However, some elements, particularly phosphorus, can only be obtained in sufficient quantities through the diet (Lall, 1991). Meeting requirements for these inorganic elements does not equate to over fortifying the feeds with them since excess will be excreted and which has been shown to have detrimental environmental effects (Davis and Gatlin, 1996).

The current study aimed to measure the crude chemical, mineral and trace element composition of cultured striped trumpeter throughout their life cycle in captivity by analysing fish collected from production runs conducted at the Marine Research Laboratories, Tasmania (MRL). Fish were reared under optimum conditions and were fed to satiation. Results from this study will provide a baseline target for future husbandry and nutrition research on this species. The specimens collected as part of this study were reared under what is currently considered to be optimum conditions; they were fed to satiation and will have the highest fat levels and energy reserves.

6.3 Materials and methods

6.3.1 *Production protocol for striped trumpeter at MRL*

Detailed descriptions of systems and rearing protocols are reviewed by Battaglione and Cobcroft (2007), but a brief overview of the protocols follows. The production of larvae and juveniles was conducted using gametes manually stripped from two groups of captive broodstock, one group of broodstock was held at ambient

temperatures while another group was maintained on a controlled temperature and photoperiod regime to ensure a constant supply of eggs (Morehead et al., 2000; Morehead et al., 2001). Eggs were incubated at 14 °C in 250 L upwelling tanks and hatch out at 84 degree days (Morehead and Hart, 2003). Seawater used for larval rearing was ozonated and treated to remove harmful by products of ozonation (Smith et al., 2006). Larvae were reared at 16 °C in 3000 L tanks in clear water for the first five days after hatching until mouth opening. A green water environment was used and larvae were fed with rotifers (*Brachionus plicatilis*) during first feeding at six days post-hatch (dph) (Shaw, 2006). Larvae were transferred into larger 3,000 L rathburn tanks at 16 dph and enriched *Artemia* was introduced. Post-larvae were reared at temperatures between 12 °C and 14 °C and were co-fed with formulated diets at 40 dph and were completely weaned by 50 dph (see Chapter 2 and Chapter 3). When juveniles were over 100 g in wet weight (WW) they were transferred to 25,000 L tanks. Post-larvae were fed with successively larger pellets until they metamorphosed into juveniles. Broodstock are maintained in 25,000 L tanks under conditions described by Bransden et al., (2007). Ambient seawater supplied to the 25,000 L tanks was sand-filtered. Ambient seawater temperatures from January 2006 to December 2008 are shown in Fig. 6.1. Formulated diets and their respective chemical compositions used during production are outlined in Table 6.1.

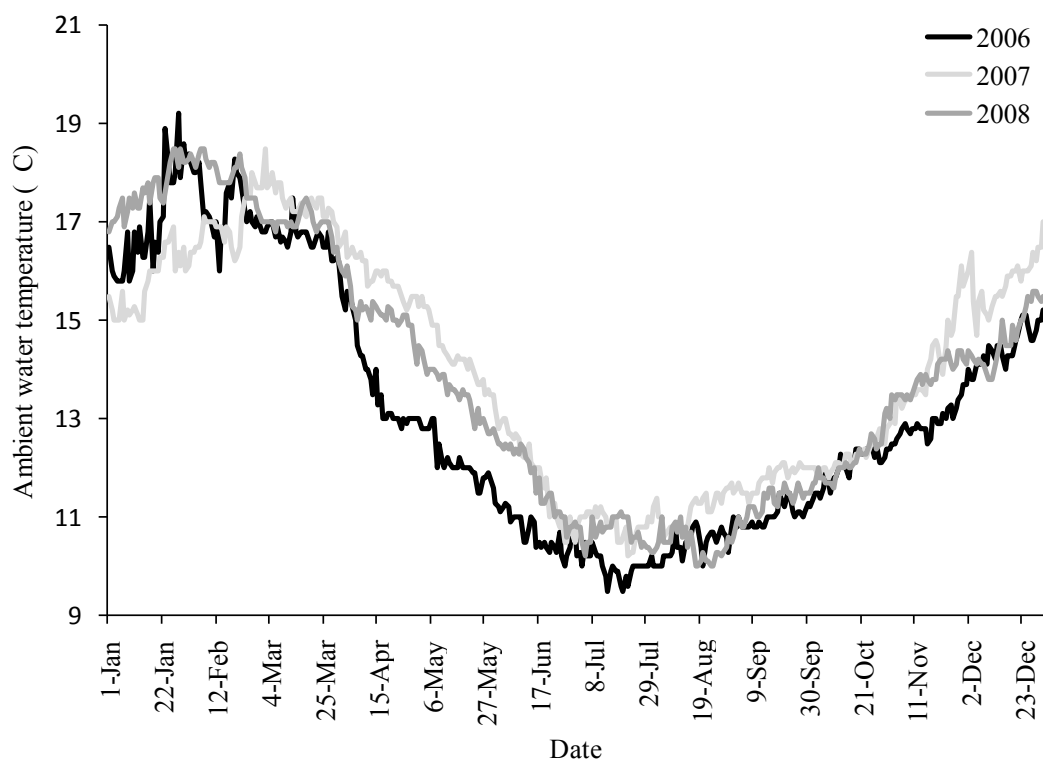


Figure 6.1: Temperature of ambient seawater supplied to 25,000 L tanks at MRL for the years 2006, 2007 and 2008.

Table 6.1: Chemical composition of the production diets fed to striped trumpeter reared at the MRL, Tasmania. All of the diets are sourced from Skretting, Australia.

Diet	Pellet Size (mm)	Fish size (g)	Crude Protein (% WW)	Total Lipid (%WW)	Energy (kJ g ⁻¹ WW)	Ash (% WW)	Moisture (% WW)
Gemma PG TM	0.8	< 1 g	53.6	22.2	22.3	9.4	8.0
Gemma PG TM	1.2	< 5g	49.3	24.3	23.0	8.4	6.9
Nutra TP TM	1.5	< 50 g	48.1	25.9	22.8	10.5	7.2
Nutra TP TM	2.0	< 100 g	47.9	26.2	22.4	10.5	7.2
Nova ME TM	3.0	< 200 g	47.0	20.8	21.7	9.1	7.0
Nova ME TM	4.0	< 500 g	46.6	20.7	21.6	10.1	6.3
Nova ME TM	6.0	< 2000 g	40.5	23.5	20.2	8.3	5.0
Nova ME TM	9.0	Broodstock	41.3	25.0	22.5	7.8	5.3
Vitalis SA TM	9.0	Broodstock	38.2	25.2	21.1	8.5	7.7

6.3.2 Sampling protocol

Samples of striped trumpeter post-larvae, juveniles and sexually mature male and female broodstock were collected. Fish were sampled every time they doubled in weight (1, 2, 4, 8, 16, 32, 64, 120, 250, 500 and 1000 g). Five replicate samples of five fish each were taken for 1 and 2 g fish. Five replicate samples of four fish were taken for 4 g fish. Five individual fish were sampled for fish of 8, 16, 32, 64, 120, 250, 500 and 1000 g. In addition, three male ($1,749.6 \pm 152.2$ g, mean \pm SE) and three female ($1,501.7 \pm 236.3$ g, mean \pm SE) F1 broodstock were collected. The broodstock specimens were transferred to a 10,000 L tank for observation of gonad maturity. Sexual maturity of female broodstock was assessed by taking a sample of eggs using a catheter and examining the percentage of hydrated eggs. Females were euthanased when the biopsies showed that $> 90\%$ of the eggs were hydrated. Male broodstock were euthanased when they were freely releasing milt. All of the fish sampled were starved for 24 h prior to euthanasia with a dose of 5 ml in 20 L seawater of Aquí-S™ (Aquí-S, New Zealand). The fish sampled for this study had slight jaw malformations but other than that exhibited normal skeletal development. All of the procedures used in this study were granted approval by the Animal Ethics Committee of the University of Tasmania (Approval number A0008719).

6.3.3 Chemical analysis

Samples were frozen and kept at $-20\text{ }^{\circ}\text{C}$ prior to analysis. Samples were autoclaved and freeze dried to a constant weight prior to analysis (Williams et al., 1995). The dry weight of the commercial extruded diet samples and individual fish

were determined by freeze drying to a constant weight. Freeze dried samples of the diet and individual fish were ground using a ceramic mortar and pestle to a homogenous powder prior to biochemical analysis. For fish up to 120 g in size, crude protein was determined using an elemental analyser (Thermo Finnigan 1112 Series elemental analyser, $N \times 6.25$); crude protein of samples 250 g and above was determined using Kjeldahl ($N \times 6.25$); total lipid was determined using the method of Bligh and Dyer (1959); and ash was determined by combustion at 550 °C for 6 h . Energy content was calculated using conversion factors for protein (23.6 kJ g⁻¹) and lipids (36.2 kJ g⁻¹) (Brafield, 1985). Energy content of the diets was determined using a bomb calorimeter. Three replicate measurements for crude protein, total lipid and two replicate measurements for ash were performed for each sample. All composition data are presented as a mean percentage of the WW of each individual fish sampled from each treatment (Shearer et al., 1994).

6.3.4 *Elemental analysis*

An equal amount of homogenate from each sample of each weight class was weighed and pooled. Two replicate samples of 0.4 g of each pooled homogenate from each weight class were acid digested at 100 °C with 10 mL of nitric acid (Aristar Grade, 16 M HNO₃), left to cool at room temperature and 10 mL of hydrogen peroxide (30% w/v) added and again heated to 100 °C. After decomposition of all of the material in the test tube, purified, de-ionized water was added to make up the volume to 50 mL. Samples were analysed for mineral content using inductively coupled plasma optical emission spectrophotometry (Thermo Jarell-Ash IRIS Axial ICP-OES). Blank samples were provided for calibration of

the ICP-OES machine. The ICP-OES machine was calibrated using known standards (Multi-Element Standard, ICPM0143-5; EM Sciences, Gibbstown, NJ, USA). Results from the ICP-OES measurement were expressed in terms of parts per million (ppm), these were converted into percentages and multiplied by the known mean dry mass of each sample to arrive at an absolute amount for each element in the whole body.

6.3.5 *Calculations and statistical analysis*

The condition (k) calculated as: $(WW / \text{Total length}^3) \times 100$ (Weatherley and Gill, 1987). Chemical composition data is presented as a percentage of WW (% WW) (Shearer, et al., 1994). A power function was used to determine significant relationships between WW and condition (k) and crude protein, total lipid, gross energy, moisture and ash. Linear regressions and power functions were used to determine significant relationships between condition and crude protein, total lipid, gross energy, moisture and ash. Elemental composition data were plotted on a log WW (g)-log element (amount) basis and a power function was fitted to determine significant relationships. A t -test was used to compare mean chemical composition of post-larvae and juveniles. All results are reported as mean \pm SE. Statistical significance was accepted at $p \leq 0.05$. Statistical analyses were performed using SPSS version 15.0 statistical software.

6.4. Results

6.4.1 *Chemical composition of growth*

Samples were collected during production runs of striped trumpeter at the MRL, Tasmania from the years 2007 to 2008. The whole body chemical composition of striped trumpeter from 1 to 1000 g is shown in Fig. 6.2. The relationship between crude protein, total lipid, ash, moisture and gross energy content to WW was fitted using a power function. Crude protein content did not change with increasing WW ($r^2 = 0.147$, $F = 9.1$). Overall crude protein content of striped trumpeter from 1 to 1000 g was 17.8 ± 0.1 % of WW. Ash content also did not change with increasing WW ($r^2 = 0.003$, $F = 0.2$). Overall ash content of striped trumpeter from 1 g to 1000 g was 4.5 ± 0.2 % of WW. A significant relationship between total lipid, moisture and energy content and WW was found in striped trumpeter from 1 g to 1000 g. A significant inverse relationship between total lipid (% WW) and moisture content (% WW) was also found ($r^2 = 0.967$, $F = 1711.9$, $p < 0.001$) (Fig. 6.3)

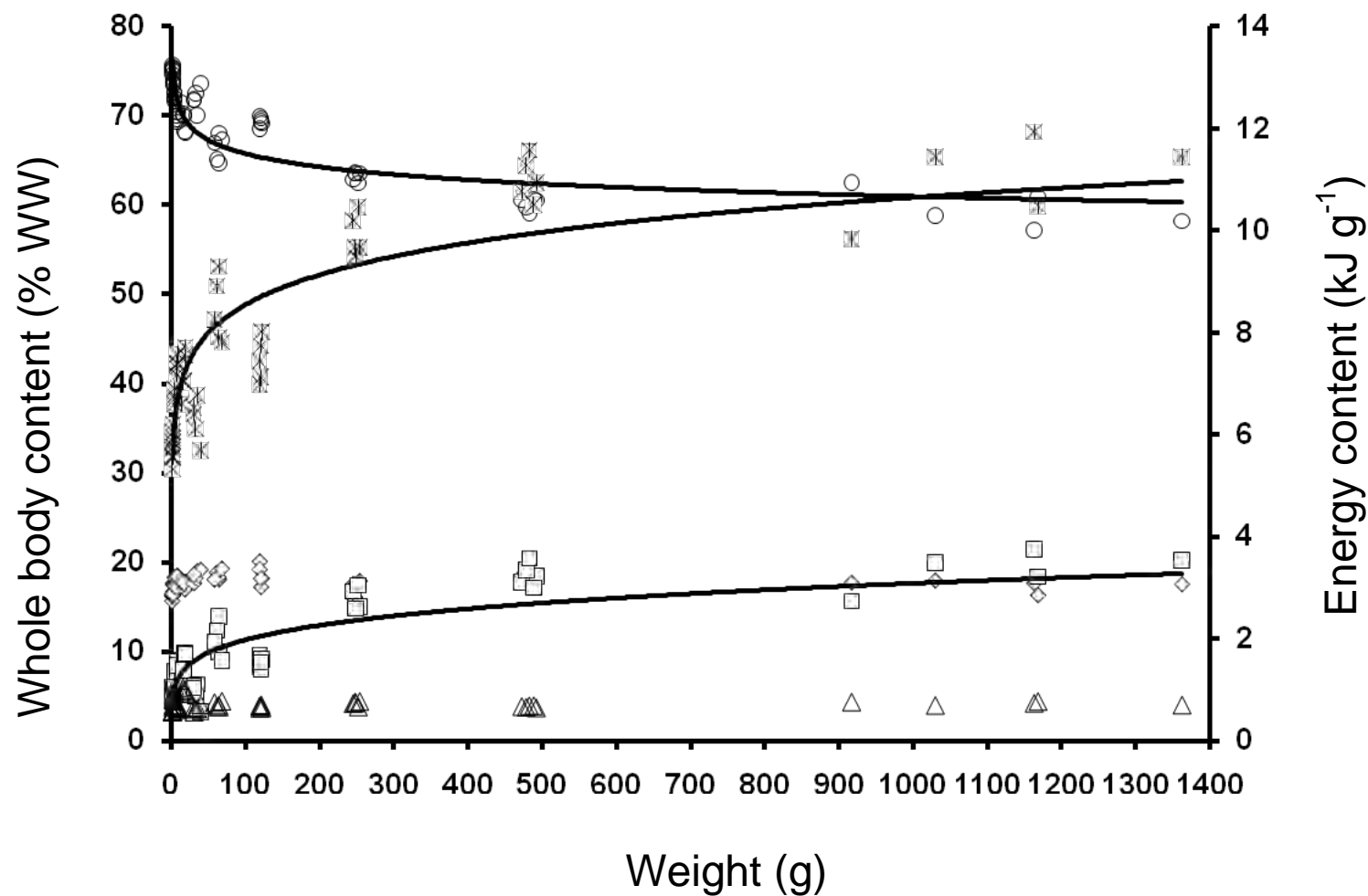


Figure 6.2: Whole body chemical composition (% WW and kJ g⁻¹) of cultured striped trumpeter from 1 g to 1000 g. Each data point represents one replicate sample. Crude protein is represented by (◇), total lipid is represented by (□), ash is represented by (Δ), moisture is represented by (○) and gross energy content is represented by (*). Only significant allometric relationships were drawn. Total lipid (% WW) = $4.7 \times WW^{0.2}$ ($r^2 = 0.730$, $F = 143.4$, $p < 0.001$); Moisture (% WW) = $76.4 \times WW^{-0.3}$ ($r^2 = 0.832$, $F = 263.2$, $p < 0.001$); Energy (kJ g⁻¹) = $5.5 \times WW^{0.9}$ ($r^2 = 0.795$, $F = 206.1$, $p < 0.001$).

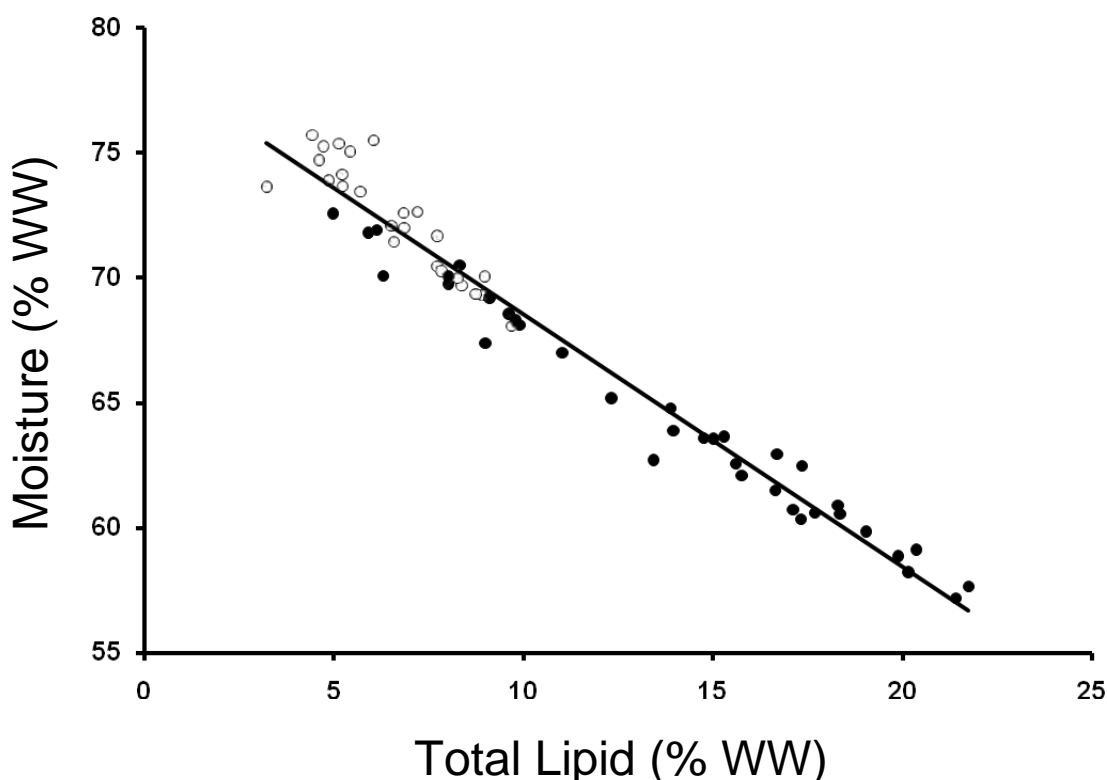


Figure 6.3: Inverse relationship between total lipid and moisture content of cultured striped trumpeter throughout their life cycle (1 to 1000 g). Measurements shown as open circles represent post-larval measurements. The linear equation for the relationship is: Moisture content (% WW) = $-1.01 \times \text{Total lipid (\% WW)} + 0.79$ ($r^2 = 0.967$, $F = 1,710.5$, $p < 0.001$)

6.4.2 Comparison of post-larvae and juveniles

Specimens collected were classified as post-larvae if they did not possess the characteristic colouration of juvenile striped trumpeter. Fish from 1 to 16 g in wet weight were classified as post-larvae and fish from 32 to 1000 g in wet weight were classified as juveniles. The crude protein content of post-larvae and juveniles (17.7 ± 0.1 % WW) were not significantly different ($df = 52$, $F = 0.2$, $p = 0.655$). Differences in chemical composition of post-larvae and juveniles were observed for total lipid, moisture, ash, and energy carcass content. Total lipid of post-larvae (6.8 ± 0.3 %

WW) was significantly lower than total lipid content of juveniles (13.3 ± 1.0 % WW) ($df = 52$, $F = 43.3$, $p < 0.001$). These results are reflected in the significantly lower energy content of post-larvae (6.6 ± 0.1 kJ g⁻¹ WW) compared to juveniles (9.1 ± 0.3 kJ g⁻¹ WW) ($df = 52$, $F = 27.9$, $p < 0.001$). Moisture content of post-larvae (72.2 ± 0.5 % WW) was significantly higher than that of juveniles (65.0 ± 0.9 % WW) ($df = 52$, $F = 17.5$, $p < 0.001$). Ash content of post-larvae (4.4 ± 0.2 % WW) was also significantly higher than that of juveniles (3.9 ± 0.1 % WW) ($df = 52$, $F = 54.9$, $p < 0.001$).

The condition (k) of post-larvae (0.7 ± 0.0) was significantly lower than the condition of juveniles (1.1 ± 0.0) ($df = 52$, $F = 6.8$, $p = 0.012$). Condition was used as a predictor for chemical composition of post-larvae and juveniles. A significant but weak relationship was found between condition (k) and crude protein content of post-larvae and juveniles ($r^2 = 0.113$, $F = 6.6$, $p = 0.013$). No significant relationship was found between condition (k) and ash content of post-larvae ($r^2 = 0.148$, $F = 4.0$, $p = 0.058$). A significant but weak relationship was found between k and ash content of juveniles ($r^2 = 0.252$, $F = 9.1$, $p = 0.005$). Significant relationships between condition (k) and total lipid, moisture and energy content were found as shown in Figs. 6.4 and 6.5. Relationships between condition (k) of post-larvae and total lipid, moisture and energy were best described by linear regressions while those of juveniles were best described by power functions.

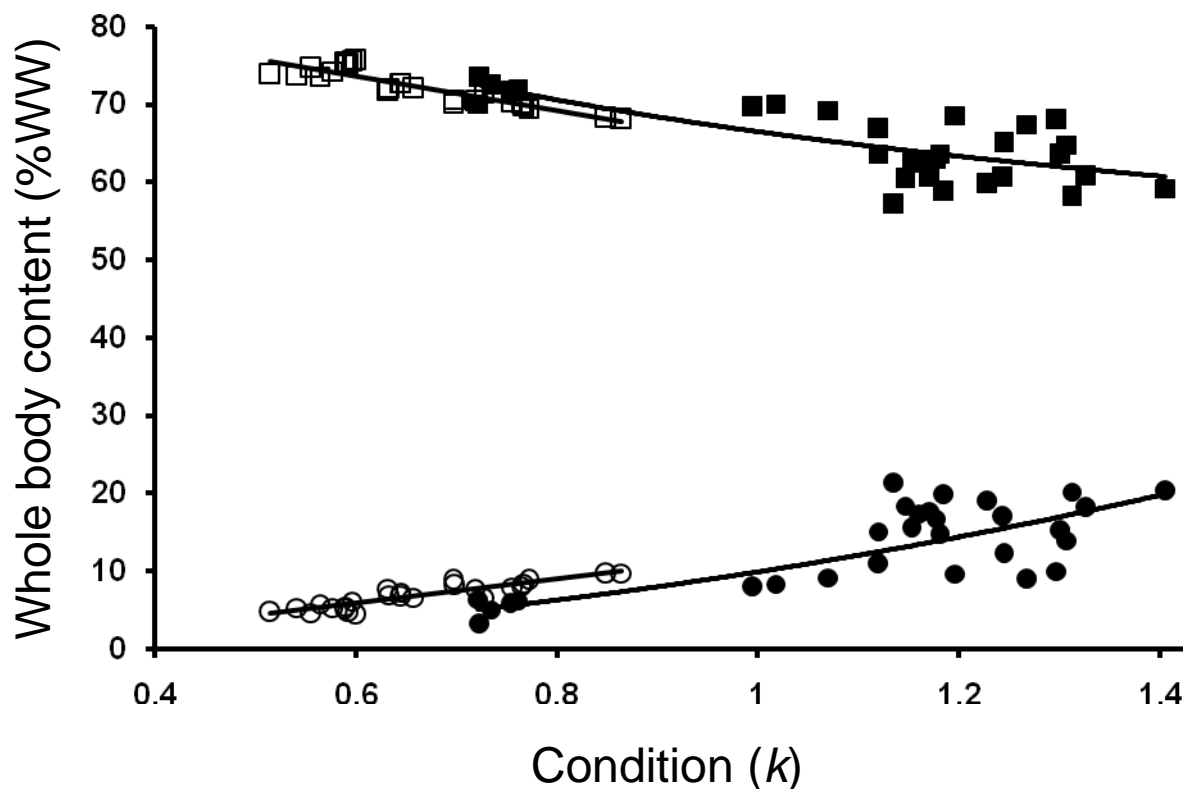


Figure 6.4: Relationship between k and whole body total lipid of post-larvae (\circ) (Total lipid % WW = $15.6 \times k - 3.5$, $r^2 = 0.811$, $F = 98.5$, $p < 0.001$) and juveniles (\bullet) (Total lipid % WW = $9.9 \times k^{2.1}$, $r^2 = 0.691$, $F = 60.5$, $p < 0.001$); and the relationship between moisture content of post-larvae (\square) (Moisture % WW = $-22.3 \times k + 87.0$, $r^2 = 0.813$, $F = 99.9$, $p < 0.001$) and juveniles (\blacksquare) (Moisture % WW = $66.5 \times k^{-0.3}$, $r^2 = 0.534$, $F = 66.5$, $p < 0.001$).

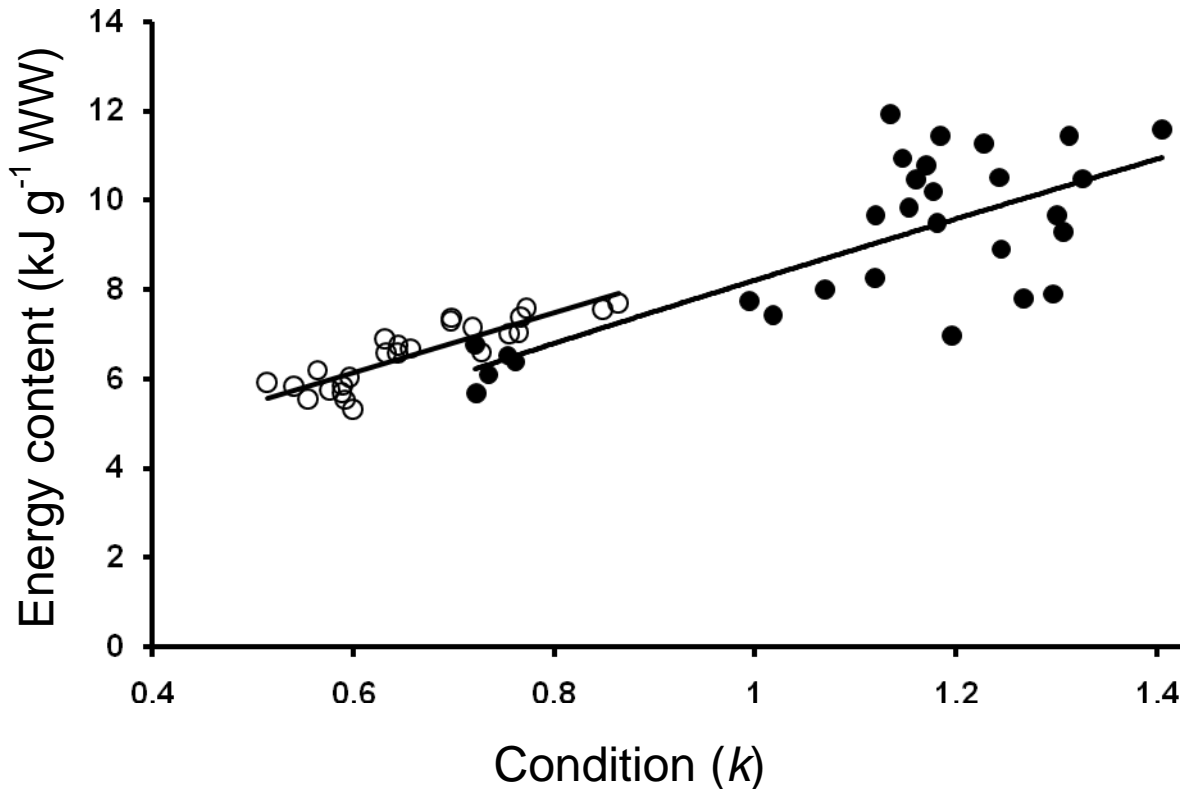


Figure 6.5: Relationship between k and energy content of post-larvae (\circ) (Energy content $\text{kJ g}^{-1} \text{WW} = 6.8 \times k + 2.1$, $r^2 = 0.766$, $F = 75.3$, $p < 0.001$) and juveniles (\bullet) (Energy content $\text{kJ g}^{-1} \text{WW} = 8.2 \times k^{0.9}$, $r^2 = 0.608$, $F = 41.8$, $p < 0.001$).

6.4.3 Mineral and trace element composition

The mineral and trace element composition of striped trumpeter post-larvae and juveniles showed a significant relationship with WW which were best described using a power function. Mineral and trace element composition of post-larvae are presented in Table 6.2 and the mineral and trace element composition of juveniles are presented in Table 6.3.

Table 6.2: Mineral (mg) and trace element (μg) composition of striped trumpeter post-larvae (1 to 16 g) reared at the MRL, Tasmania fitted with a power function.

Element (Amount)	r^2	F	p	Amount = $a \text{ WW (g)}^b$	
				a	b
Minerals					
Calcium (mg)	0.998	4,055.6	<0.001	1.656	1.135
Magnesium (mg)	0.994	1,344.0	<0.001	0.089	1.101
Phosphorus (mg)	0.999	12,002.9	<0.001	1.242	1.127
Potassium (mg)	0.999	11,580.4	<0.001	0.794	1.114
Sodium (mg)	0.985	527.3	<0.001	0.387	1.098
Strontium (mg)	1.000	36,527.5	<0.001	0.005	1.144
Trace elements					
Aluminium (μg)	0.946	140.1	<0.001	0.651	1.007
Barium (μg)	0.951	156.0	<0.001	0.020	1.134
Caesium (μg)	0.969	248.1	<0.001	0.003	1.046
Chromium (μg)	0.814	35.1	<0.001	0.014	1.406
Copper (μg)	0.989	694.3	<0.001	0.231	1.036
Iron (μg)	0.998	4,573.7	<0.001	3.488	1.092
Lead (μg)	0.980	401.3	<0.001	0.006	0.987
Manganese (μg)	0.997	2,388.8	<0.001	0.372	1.087
Rubidium (μg)	0.999	6,347.4	<0.001	0.145	1.109
Zinc (μg)	0.998	4,777.5	<0.001	5.384	1.011
Cadmium (μg)	0.945	137.4	<0.001	.001	1.225
Cobalt (μg)	0.965	218.0	<0.001	0.002	1.001
Thallium (μg)	0.644	14.5	.005	0.00004	1.723
Uranium (μg)	0.908	78.9	<0.001	.001	1.075
Vanadium (μg)	0.798	31.5	.001	.003	.821

Table 6.3: Mineral (mg) and trace element (μg) composition of striped trumpeter juveniles (32 to 1000 g) reared at the MRL, Tasmania fitted with a power function.

Element (Amount)	r^2	F	p	Amount = $a \text{ WW (g)}^b$	
				a	b
Minerals					
Calcium (mg)	0.993	1,374.5	<0.001	1.633	1.141
Magnesium (mg)	0.995	1,851.7	<0.001	0.072	1.108
Phosphorus (mg)	0.995	1,910.1	<0.001	1.243	1.115
Potassium (mg)	0.995	2,034.5	<0.001	0.860	1.054
Sodium (mg)	0.992	1,236.2	<0.001	0.317	1.037
Strontium (mg)	0.992	1,270.8	<0.001	.003	1.216
Trace elements					
Aluminium (μg)	0.920	115.6	<0.001	0.589	1.035
Barium (μg)	0.983	575.5	<0.001	.009	1.363
Caesium (μg)	0.988	830.6	<0.001	.001	1.255
Chromium (μg)	0.894	84.3	<0.001	0.002	1.331
Copper (μg)	0.978	450.2	<0.001	0.119	1.093
Iron (μg)	0.988	834.9	<0.001	1.921	1.187
Lead (μg)	0.929	131.8	<0.001	.002	1.351
Manganese (μg)	0.981	520.6	<0.001	0.321	1.017
Rubidium (μg)	0.997	2,864.9	<0.001	0.116	1.136
Zinc (μg)	0.995	2,099.1	<0.001	2.950	1.050
Cadmium (μg)	0.979	459.6	<0.001	0.002	0.908
Cobalt (μg)	0.987	783.0	<0.001	0.002	1.046
Thallium (μg)	0.429	5.2	<0.001	.002	.326
Uranium (μg)	0.952	198.8	<0.001	0.0002	1.299
Vanadium (μg)	0.980	502.0	<0.001	0.012	.849

6.4.4 Composition of broodstock and eggs

The whole body chemical compositions of male and female broodstock are presented in Table 6.4. The elemental composition of male and female broodstock and eggs are presented in Table 6.5. Eggs collected from the three female broodstock measured $1.1 \pm 0.1 \text{ mm}^3$ ($n = 30$) in volume. Eggs were composed of $14.7 \pm 0.3 \%$ WW crude protein, $8.2 \pm 1.5 \%$ WW total lipid, $2.3 \pm 0.1 \%$ WW ash, $74.9 \pm 1.6 \%$ WW moisture and an energy content of $6.4 \pm 0.6 \text{ kJ g}^{-1}$.

Table 6.4: Whole body chemical composition of male and female broodstock reared at the MRL, Tasmania.

	Broodstock (male)	Broodstock (female)
Wet weight (g)	$1,749.6 \pm 152.2$	$1,501.7 \pm 236.3$
Length (cm)	531.7 ± 17.6	501.7 ± 19.6
Gonad weight (g)	17.3 ± 6.2	85.0 ± 28.5
Gonadosomatic index	1.1 ± 0.0	5.4 ± 0.0
Crude protein (% WW)	18.0 ± 0.8	18.2 ± 0.3
Total lipid (%WW)	16.4 ± 2.7	16.6 ± 0.5
Ash (% WW)	4.9 ± 0.6	4.77 ± 0.0
Moisture (% WW)	61.4 ± 2.0	61.3 ± 0.5
Energy (kJ g^{-1} WW)	10.1 ± 0.9	10.3 ± 0.2

Table 6.5: Mineral and trace element composition of male and female broodstock striped trumpeter and eggs reared at the MRL, Tasmania.

Element (ppm, wet basis)	Broodstock (Male)	Broodstock (Female)	Eggs
Minerals			
Calcium	5,340.98 \pm 402.63	4,806.08 \pm 89.08	39.57 \pm 7.85
Magnesium	138.73 \pm 4.11	129.84 \pm 2.38	38.74 \pm 3.24
Phosphorus	3,136.61 \pm 187.57	2,671.99 \pm 48.50	741.09 \pm 33.10
Potassium	1,171.25 \pm 21.91	1,097.81 \pm 38.80	796.09 \pm 118.39
Sodium	408.49 \pm 26.44	345.68 \pm 16.32	368.81 \pm 54.80
Strontium	19.25 \pm 1.64	17.59 \pm 0.42	0.19 \pm 0.04
Trace elements			
Aluminium	1.05 \pm 0.04	0.80 \pm 0.08	0.35 \pm 0.05
Barium	0.12 \pm 0.01	0.11 \pm 0.01	0.003 \pm 0.002
Caesium	0.01 \pm 0.00	0.01 \pm 0.00	0.003 \pm 0.000
Chromium	0.02 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
Copper	0.46 \pm 0.14	0.12 \pm 0.01	0.30 \pm 0.02
Iron	5.68 \pm 0.22	3.61 \pm 0.58	2.14 \pm 0.15
Lead	0.02 \pm 0.00	0.02 \pm 0.00	0.003 \pm 0.001
Manganese	0.42 \pm 0.02	0.36 \pm 0.01	0.24 \pm 0.03
Rubidium	0.22 \pm 0.02	0.21 \pm 0.01	0.17 \pm 0.03
Zinc	3.95 \pm 0.02	3.85 \pm 0.16	13.30 \pm 0.88
Cadmium	0.0025 \pm 0.0004	0.0021 \pm 0.0005	0.0002 \pm 0.0001
Cobalt	0.0023 \pm 0.0002	0.0012 \pm 0.0002	0.0060 \pm 0.0006
Thallium	0.0006 \pm 0.0004	0.0001 \pm 0.0000	0.0001 \pm 0.0000
Uranium	0.0025 \pm 0.0005	0.0021 \pm 0.0003	0.0003 \pm 0.0001
Vanadium	0.0070 \pm 0.0011	0.0052 \pm 0.0005	0.0009 \pm 0.0001

6.5 Discussion

This study provided the first empirical information on the changes in crude chemical, mineral and trace element composition of striped trumpeter post-larvae, juveniles, broodstock and eggs. The chemical composition of striped trumpeter followed similar trends to those observed in studies on Atlantic salmon (*Salmo salar*) (Shearer, et al., 1994), sea bream (*Sparus aurata*) (Lupatsch et al., 1998); European sea bass (*Dicentrarchus labrax*) (Lupatsch et al., 2001) and sharpsnout bream (*Diplodus puntazzo*) (Hernandez et al., 2003). Crude protein and ash content remained constant as fish grew from post-larvae to juveniles while total lipid increased and moisture decreased, consequently energy content also increased as fish grew.

Significant relationships between total lipid, moisture content and energy were found (Fig. 6.2) and these equations were applied to commercially important milestones for striped trumpeter culture (Fig. 6.6). The estimated chemical composition of striped trumpeter at these growth stanzas are compared with the chemical composition of similar commercially cultured temperate marine species, sea bream (*Sparus aurata*) (Lupatsch et al., 1998) and European sea bass (*Dicentrarchus labrax*) (Lupatsch et al., 2001) (Table 6.6). The chemical compositions of all three temperate species were very similar to each other. Crude protein content of striped trumpeter and sea bream are similar, sea bream had the highest total lipid content, followed by striped trumpeter and sea bass had the lowest. The energy content of striped trumpeter is similar to European sea bass and sea bream had the highest energy content.

Cultured fish generally have higher fat content compared to their wild counterparts (Alasalvar et al., 2002; Cahu et al., 2004). Cultured striped trumpeter is considered to be a fatty fish (> 10 % fat) (Yearsley et al., 1999; Colquhoun et al., 2008) but more importantly it had the highest concentration of *n*-3 polyunsaturated fatty acids globally recorded for an aquaculture species (Nichols et al., 2005). The *n*-3 polyunsaturated fatty acids are important constituents of a healthy diet to promote cardiovascular health, development of unborn children, autoimmune disorders and prevention of other diseases (Connor, 2000; Kris-Etherton et al., 2002). The increase in fat content is attributable to the diets used for rearing commercial species. The high fat content of fish fillets can be a positive or a negative, for example a high fat content in fish flesh contributes to a shorter shelf-life for the product (Sivertsvik et al., 2002) but salmon fillets with a high fat content improved the taste properties during cold smoking (Morkore et al., 2001).



1. Early post-larvae (10 g)



2. Beginning of metamorphosis into juveniles (30 g)



**3. Completion of metamorphosis into juvenile;
ready for stocking into sea cages (50 g)**



4. Juvenile (250 g)



5. Market size (1000 g)

Figure 6.6: Specimens of striped trumpeter at growth stanzas for commercial culture and the approximate wet weight of fish at these stages. The chemical composition of striped trumpeter at these stages is detailed in Table 6.6.

Table 6.6 Estimated whole body chemical composition of striped trumpeter at selected growth stanzas (Fig. 6.6) compared with the estimated chemical composition of European sea bass (Lupatsch et al., 2001) and sea bream (Lupatsch et al., 1998) at the same sizes.

	1	2	3	4	5
	(~10 g)	(~30 g)	(~50 g)	(~250 g)	(~1000 g)
Striped trumpeter					
Crude protein (g)	1.8	5.3	8.9	44.5	177.8
Total lipid (g)	0.7	2.8	5.1	35.3	186.3
Total energy (kJ)	69.0	228.0	400	2,325.0	10,700.0
Moisture (g)	7.1	20.7	34.0	161.9	621.2
Ash (g)	0.4	1.3	2.1	10.3	39.7
European sea bass					
Crude protein (g)	1.7	5.1	8.6	42.8	171.0
Total lipid (g)	0.8	2.8	5.1	34.0	173.8
Total energy (kJ)	103.0	312.0	520	2,650	10,700.0
Moisture (g)	7.0	20.5	33.7	161.3	621.3
Ash (g)	0.5	1.4	2.3	11.6	46.3
Sea bream					
Crude protein (g)	1.8	5.4	9.0	44.8	179.0
Total lipid (g)	0.8	3.0	5.4	37.9	201.8
Total energy (kJ)	67.0	228.0	400.0	2,425.0	11,400.0
Moisture (g)	7.1	20.4	33.4	156.3	591.6
Ash (g)	0.5	1.4	2.3	11.3	45.0

The chemical composition of post-larvae differed from those of juveniles and supports findings from a separate growth trial (see Chapter 4). In the current life-cycle study, post-larvae possessed lower crude protein and total lipid content and higher ash and moisture content compared to juveniles. Therefore, post-larvae had lower energy content. Chemical composition results from Chapter 4 show crude protein content of post-larvae and juveniles was 16.9 % WW, total lipid content of post-larvae was 4.0 % WW and that of juveniles was 7.0 % WW. Comparing the results of the current study and Chapter 4, the crude protein content of 17.8 % WW found in the current study was slightly higher and the total lipid content for post-larvae (7.0 to 9.3 % WW) and juveniles (10.2 to 18.6 % WW) were also higher. The differences can be explained by the larger size range of juveniles sampled for the current study, up to 1000 g, and it was found that lipid deposition accelerates throughout the juvenile life history stage. Another explanation is that results from Chapter 4 were obtained under experimental conditions using an experimental diet that had a protein and energy ratio below the predicted optimum (see Chapter 4) whereas results from this study represent optimum rearing conditions. The key finding from the current study of chemical composition of post-larvae and juveniles is that important growth processes occur during the post-larval growth phase and nutrients are required to provide substrates for tissue and muscle development and fuel for growth which can account for the lower energetic reserves of post-larvae. Once post-larvae complete metamorphosis into juveniles, accretion of total lipid and energy accelerates. These findings are also supported by the relationship of chemical composition with condition. Condition and chemical composition equations found in this study can be used for rapid assessment of nutrient accretion in striped trumpeter.

The chemical composition of sexually mature broodstock is also presented. Bransden et al., (2007) used a Torry Fish Fatmeter to measure changes in broodstock condition during the spawning season. They found that males lose 25 % of muscle fat and females lose 40 % of muscle fat during gonadogenesis. Bransden et al., (2007) placed emphasis on the total lipid content of the eggs because of the important role of lipids in providing energy to the developing embryo and larva and material for tissue and membrane development (Sargent, 1995; Sargent et al., 2002). A total lipid content of 2 % of wet weight for striped trumpeter eggs found in the current study is similar to the findings of Morehead et al., (2001). Results of the current study indicate that broodstock nutrition of striped trumpeter is managed successfully using “best practice” procedures (Battaglione and Cobcroft, 2007).

Mineral and trace element composition can provide information about requirements. Phosphorus status is determined by the amount consumed and up to the optimum dietary inclusion level phosphorus status will increase after which homeostatic mechanisms will regulate its deposition and whole body content (Rodehutscord et al., 2000). Information on the phosphorus composition of fish has the potential to provide useful information about their requirement status. Pimentel-Rodrigues and Oliva-Teles (2001) investigated phosphorus requirement of sea bream (5.0 g) and another study by Oliva-Teles and Pimentel-Rodrigues (2004) investigated phosphorus requirement of European sea bass (10.0 g). The mean phosphorus content of sea bream juveniles was 0.5 g (0.5 % WW) and was 1.5 g (0.7 % WW) in sea bass juveniles. The mean phosphorus content of striped trumpeter post-larvae was considerably less at 0.15 % WW and in juveniles was 0.23 % WW. The difference in phosphorus content appears related to inter-species differences of skeletal tissues and scales, which is the primary repository of phosphorus in fish

(Lall, 2002). These studies found that phosphorus inclusion beyond the requirement level did not increase phosphorus content of the fish. It is reasonable to assume that this will hold true for other minerals and trace elements which are primarily derived from dietary sources (zinc, manganese, magnesium, iron, copper, selenium, iodine, fluoride) (NRC, 1993).

The development of jaw malformations in striped trumpeter is described by Cobcroft et al., (2001). Jaw malformations develop during the larval growth phase and the effect of the tank colour and lighting dynamics have been implicated as causes (Cobcroft and Battaglione, 2009). Future research on preventing jaw malformations could also investigate the requirements for these inorganic elements, through phospholipid supplementation, during larval growth (Cobcroft et al., 2001). Other than slight jaw malformations in the sampled fish, there were no other physical signs which indicated a mineral or trace element deficiency. Other skeletal deformities caused by a deficiency in minerals are curved or enlarged vertebrae, deformed spines and heads, shortened operculum, and scoliosis (Lall, 2002; Roy and Lall, 2003; Lall and Lewis-McCrea, 2007). Other less overt signals of mineral and trace element deficiencies are reduced growth and bone mineralization, degeneration of muscle fibres, skin erosion, abnormal tail growth and convulsions (NRC, 1993; Lall, 2002). The estimated mineral and trace element composition of striped trumpeter at commercially important growth stanzas are presented in Table 6.7.

Table 6.7 Estimated mineral and trace element composition of striped trumpeter at important growth stanzas. Equations used to estimate composition are found in Tables 6.2 and 6.3.

	1	2	3	4	5
	(~10 g)	(~30 g)	(~50 g)	(~250 g)	(~1000 g)
Minerals					
Calcium (mg)	22.60	78.63	141.75	889.27	4,325.0
Magnesium (mg)	1.12	3.76	5.49	32.68	151.8
Phosphorus (mg)	16.64	57.39	97.46	586.37	2750.9
Potassium (mg)	10.32	35.10	53.11	289.69	1248.82
Sodium (mg)	4.85	16.20	18.32	97.21	409.32
Strontium (mg)	0.07	0.24	0.35	2.47	13.34
Trace elements					
Aluminium (µg)	6.62	20.00	33.77	178.64	750.09
Barium (µg)	0.27	0.95	1.86	16.70	110.47
Caesium (µg)	0.03	0.11	0.14	1.02	5.82
Chromium (µg)	0.36	1.67	0.37	3.11	19.68
Copper (µg)	2.51	7.83	8.56	49.72	226.23
Iron (µg)	43.11	143.08	199.62	1348.60	6990.81
Lead (µg)	0.06	0.17	0.39	3.47	22.60
Manganese (µg)	4.55	15.00	17.15	88.15	361.00
Rubidium (µg)	1.86	6.30	9.87	61.45	296.80
Zinc (µg)	55.22	167.68	179.37	971.98	4166.69
Cadmium (µg)	0.02	0.06	0.07	0.30	1.06
Cobalt (µg)	0.02	0.06	0.12	0.64	2.75
Thallium (µg)	0.002	0.014	0.006	0.010	0.015
Uranium (µg)	0.01	0.04	0.03	0.26	1.58
Vanadium (µg)	0.02	0.05	0.33	1.30	4.23

The current study highlights the importance of differentiating between requirements for post-larvae and juvenile culture. The empirical data can be used in diet formulation and development of nutrient requirement models. This could be achieved through the addition of data on maintenance requirements and nutrient utilisation efficiency rates (Hauler and Carter, 2001). Maintenance requirements represent the minimum amount needed while nutrient accretion under optimum conditions represent the maximum amount that can be incorporated. Measuring digestibility in striped trumpeter is an identified research need (Bureau et al., 2002) but is made difficult by the sensitive nature of post-larvae preventing manual stripping while the small volume of faeces produces by post-larvae makes faeces collection complicated. However, this would be more straightforward in larger fish. Requirements for many minerals and trace elements are also difficult to identify because of the need to rear fish in water devoid of the element of interest (Lall, 2002). However, there are some minerals for which requirements and utilisation have been studied such as phosphorus (Sajjadi and Carter, 2004). Consequently, in the current situation baseline data would be best applied as target amounts for normal growth in striped trumpeter.

Striped trumpeter is very similar to European sea bass and sea bream in its chemical composition. Sea bass reach market size of (450 g) in 20 to 24 months but this is reduced to 14 to 15 months in warmer waters; requires 43 to 50 % protein and 12 to 25 % fat in the diet (Jobling et al., 2009). Sea bream reach market size (350 g) in 20 months; requires 45 to 50 % protein, 20 % fat and 20 % carbohydrates in their diet (Jobling and Peruzzi, 2009). Commercial production for sea bass and sea bream was achieved after intensive hatchery methods were developed in the 1970s for sea bass and in the 1980s sea bream and the subsequent investment in hatchery and

grow-out operations. These similarities further highlight the potential of striped trumpeter to emerge as a valuable addition to the Tasmanian aquaculture industry.

The identification of nutrient requirements for striped trumpeter is enhanced by findings from the current study which establishes maximum accretion of nutrients throughout the life-cycle. Understanding nutrient requirements requires knowledge about maintenance and retention. The study has established a reference for chemical, mineral and trace element composition of striped trumpeter throughout its life cycle. Differences in composition between species and between results from separate growth trials highlight the importance of baseline data as a basis for comparison.

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Chapter 7

General Discussion

7. General discussion

This study provided the first detailed research into the growth and optimal rearing conditions for striped trumpeter post-larvae. One of the most significant challenges facing striped trumpeter culture is its prolonged post-larval stage. Survival of post-larvae has been highly variable because they are susceptible to stressors and fluctuations in water quality which can lead to mortality (Battaglione and Brown, 2006; Battaglione and Cobcroft, 2007a). Previous attempts to investigate optimizing culture parameters for post-larvae were not possible because of a lack of animals. In addition, striped trumpeter post-larvae produced prior to 2005 were often infected with *myxosporeans* and other diseases and the resulting numbers produced were insufficient to conduct large scale replicated experiments (Grossel et al., 2003; Grossel, 2005; Battaglione and Morehead, 2006; Battaglione and Cobcroft, 2007b). The findings from my study have lead to immediate improvements in post-larvae culture and provide data for future nutrition research on striped trumpeter. The study was divided into five research chapters investigating: weaning of early post-larvae onto formulated diets; optimal rearing temperatures; effects of ration and dietary lipid; development of factorial models for predicting nutrient requirements; and changes in the chemical and elemental composition of striped trumpeter throughout its life cycle. The key findings of this study are:

- A protocol for weaning striped trumpeter post-larvae onto formulated diets by co-feeding at 40 days post-hatch (dph) and feeding exclusively on formulated diets at 50 dph.

- The recommended temperature regime is 14 °C during post-larval growth. Rearing striped trumpeter post-larvae at 14 °C and 16 °C resulted in the highest growth but metamorphosis into juveniles was highest at 12 °C and 14 °C. Rearing post-larvae at 18 °C is not advised because of reductions in growth, nutrient utilisation efficiencies and lower numbers of metamorphosed individuals.
- A daily ration of 4.0 % of biomass day⁻¹ using a diet with 44 % dietary protein and 19 % dietary lipid was found to support the highest growth and development.
- A predicted optimum dietary protein to energy 21.8 g protein MJ⁻¹ with a productive protein value of 29.1 %.
- A predicted maintenance requirement of 7.0 g kg^{0.8} d⁻¹ of diet. Predicted intakes of 1.8 g protein kg^{-0.7} d⁻¹ and 116.1 kJ kg^{-0.8} d⁻¹ to maintain carcass protein and energy.
- Post-larvae had lower energy and total lipid content, higher moisture and ash and similar crude protein content compared to juveniles.
- The crude protein and ash content of striped trumpeter remained constant while total lipid content was deposited at the expense of moisture through its life cycle.

This study investigated only a small fraction of aspects of nutrition of striped trumpeter post-larvae and more research is needed to further understand and optimise nutrition of this unique species. This is presented below in Section 7.5.

7.1 The use of formulated diets in striped trumpeter culture

The culture of the majority of marine finfish species relies on feeding with live feeds, usually rotifers and *Artemia* (Sorgeloos et al., 2001; Aragao et al., 2004; Olsen et al., 2004; Monroig et al., 2006). Striped trumpeter larvae and post-larvae were previously found to require high levels of n-3 polyunsaturated fatty acids (PUFA), specifically, docosahexaenoic acid (DHA; 22:6 n-3) (Bransden et al., 2005a,b). Feeding with *Artemia*, though convenient, requires enrichment with commercial products to meet the required levels of fatty acids (Sargent et al., 1997).

The use of formulated diets is advantageous because of potential savings in capital and hatchery operating costs (Le Ruyet et al., 1993) but also enables more direct manipulation of the nutritional profile of feeds and the possibility of achieving higher growth and prevention of skeletal deformities. The use of phospholipids can promote better growth due to increased digestibility and absorption compared to neutral lipids (Coutteau et al., 1997; Morais et al., 2007). More significantly, the incorporation of phospholipids in diets has the potential to prevent skeletal malformations. Diets high in phospholipid content promoted development of the digestive system and ossification in Atlantic cod (*Gadus morhua*) and European sea bass (*Dicentrarchus labrax*) (Villeneuve et al., 2007; Kjorsvik et al., 2009). Furthermore, the addition of trace elements can also prevent the development of skeletal deformities (Nguyen et al., 2008). Skeletal malformations in striped trumpeter are primarily in the form of jaw malformations (Cobcroft et al., 2001). Although the physical environment has been implicated as a cause for the development of the malformations (Cobcroft and Battaglione, 2009), the effect of nutrition (phospholipids and trace elements) has not yet been investigated. With the

development of a weaning strategy for striped trumpeter post-larvae, researchers can now test different diet formulations and manipulate components to measure effects on growth and promotion of normal skeletal development.

7.2 Post-larvae: an important life history stage

The terms post-larvae or “paperfish” are used to denote larvae which had undergone transformation following flexion. Post-larvae are fully scaled and have completed ray and fin development. Although striped trumpeter post-larvae have not yet been formally described, its appearance becomes increasingly silvery with deep and laterally compressed bodies and a prominent ventral keel, a specialization to pelagic oceanic life (Furlani and Ruwald, 1999). An extended paperfish stage is shared by the *Cheilodactylidae* or morwongs, and lasts 5 to 7 months in morwongs (Allen and Heemstra, 1976; Bruce, 1998; Ritar and Pribadi, 2006). The term post-larva is not favoured as a description for most marine fish because it is ambiguous (Fuiman and Werner, 2002). However, it is appropriate for striped trumpeter because it is so distinctly different in terms of ontogeny, morphology, behaviour and also because of its long duration of 9 to 10 months. Post-larvae have fully formed organs and skeletal systems but have not undergone the metamorphosis into a juvenile which is followed by settlement into rocky shore habitat (Tracey and Lyle, 2005).

In addition to morphological and behavioural differences, the chemical composition of post-larvae was found in the present study to differ from that of juveniles. Total lipid content of post-larvae was lower compared to juveniles and resulted in higher ash and moisture content (see Chapter 4 and Chapter 6). Crude protein content of post-larvae and juveniles were similar, however. Therefore, post-

larvae have a low carcass energy content. Post-larvae are delicate animals and do not tolerate stressors such as handling, changes in water quality and low dissolved oxygen levels which limited the use of techniques that required handling such as faecal stripping. This is discussed further below (see Section 7.4).

7.3 Metamorphosis into juveniles

Juveniles are characterised as being adult in appearance but are not sexually mature, exhibit an isometric growth pattern, and can also be different in their behaviour (Balon, 1999). In this study, striped trumpeter juveniles were observed to behave differently compared to post-larvae, they become more benthic-orientated and were less active which would confirm that their behaviours and morphology are suited to the rocky reefs where they are typically found (Long, 1995). Metamorphosis, therefore, is more than a change in form. Metamorphosis encompasses changes of anatomy, physiology, behavior and ecology (Wald, 1981).

This is the first study to investigate culture parameters which have an influence on the unusual metamorphosis of striped trumpeter post-larvae to juveniles. Other teleost species which undergo secondary metamorphosis are salmonids and eels and while they are not completely analogous to the metamorphosis of striped trumpeter post-larvae they can provide insights into the requirements for metamorphosis. In my study, development of post-larvae into juveniles was measured using a discrete scale based on visual appearance (see Chapter 3). Metamorphosis of striped trumpeter juveniles was found to be influenced by rearing temperature regime and food availability. The progression of metamorphosis is variable and culture conditions were found to have a profound effect on the time it

takes to complete metamorphosis into juveniles. This is shown in the difference in the recorded proportion of metamorphosed individuals between Chapters 3 and 4. Post-larvae were reared for 84 days in Chapter 3 versus 63 days in Chapter 4 resulting in individuals achieving larger sizes and higher numbers of metamorphosed individuals in Chapter 3. Of greater significance is the faster development of striped trumpeter at cooler temperatures in contrast to most other fish species (Nicieza and Metcalfe, 1997; Aritaki and Seikai, 2004; Green and Fisher, 2004).

Provision of a nutritionally adequate feed is also necessary to support metamorphosis into juveniles. Animals fed on a restricted ration did not show a high incidence of metamorphosis. The findings in my study indicate that metamorphosis of striped trumpeter post-larvae is initiated at a minimum size of 20 g but is also dependent on attainment of a threshold energy composition before metamorphosis is completed (see Chapter 4). This supports the hypothesis that a threshold for chemical composition must be attained as a trigger for metamorphosis. Salmon parr must exceed a set size and energy status by a certain time for transformation into a smolt (smoltification) to occur (Thorpe et al., 1998; Berrill et al., 2004). The benefits gained from reducing the duration of the striped trumpeter post-larval growth stage, higher survival and growth, outweigh the costs of ensuring post-larvae are continuously fed to satiation. Each post-larva represents a significant investment in time and resources, having been reared for a period of approximately 300 days before metamorphosis into juveniles begin.

7.4 Nutrient retention of striped trumpeter post-larvae

Nutrient retention efficiencies of striped trumpeter post-larvae in my study were lower than those observed for other species (discussed in Chapter 4 and Chapter 5). The inability to measure digestibility and feed intake accurately are potential causes of an underestimation of nutrient retention efficiency. Measurements of feed intake are important to determine the intake of nutrients and digestibility is a measure of the bioavailability of these nutrients. Feed intake was measured by siphoning and then drying uneaten pellets (see Chapter 3) and by an alternative method: direct observation and counting uneaten pellets (see Chapter 4). Direct observation provides an opportunity to observe feeding behaviour of the fish and provides information on changes in feed intake patterns (Jobling et al., 2001b). Feeding behaviour of striped trumpeter post-larvae also lead to an overestimation of feed intake due to difficulties in estimating feed wastage. Post-larvae were observed to ingest whole pellets but would regurgitate feed particles afterwards. It was not determined what portion of the pellet was ingested and which portion was expelled. The presence of jaw malformations in all of the fish was also suspected to cause aberrant feeding behaviour. Future studies into organoleptic and physical properties of the diet will be investigated to ensure that physical and chemical characteristics of the feed are appropriate (Jobling et al., 2001a). In future studies, feed intake can be measured by hand-feeding less frequently (2 meals day⁻¹) and counting uneaten pellets.

Cho and Kaushik (1990) found that feeding frequency had no effect on digestibility but a separate study by Fernandez et al. (1998) found that a higher feeding frequency lowered apparent digestibility coefficients in sea bream (*Sparus*

aurata). The influence of temperature on digestibility is confounded by its influence on feed intake, and to a lesser extent the gastric evacuation rate and the melting point of the lipid ingredients of the diet (Bendiksen et al., 2003). Digestibility in striped trumpeter post-larvae was not measured in this study because handling the fish was not practical, eliminating the use of faecal stripping techniques, further the volume of faeces produced by the post-larvae was not enough for collection by settlement (Carter and Hauler, 2000; Percival et al., 2001). Measuring digestibility of ingredients and diets in striped trumpeter post-larvae will provide essential information for improving diet formulation and can be completed in the future by either sacrificing post-larvae and collecting faeces by dissection or systems re-designing for collection by settlement.

The development of factorial models for growth of striped trumpeter predict that the maintenance protein requirement was $2.18 \text{ g protein kg}^{-0.7} \text{ d}^{-1}$ and a requirement of $14.26 \text{ g protein kg}^{-0.7} \text{ d}^{-1}$ is required for maximum growth of $19.10 \text{ g kg}^{0.8} \text{ d}^{-1}$. For commercial and nutritional reasons protein is arguably the most important macro-nutrient in fish diets. As well as meeting the amino acid requirements protein provides an energy source that marine fish, in particular, appear to require. Fish meal is the most utilised protein source for fish diets because of its high protein content and amino acid profile. However, it is becoming more expensive and less available so that inclusion need to be optimised (Stickney and McVey, 2002; Wilson, 2002). It is hypothesised that post-larvae have high metabolic costs associated with protein synthesis and the cost of protein synthesis is even higher in smaller animals; also striped trumpeter post-larvae are highly active and it is likely that a proportion of their protein intake is catabolised to fuel their energetic demands (Santinha et al., 1996; Carter and Houlihan, 2001). Results from my study

indicate that diets high in protein resulted in higher growth; post-larvae fed with a diet with 48.4 % (22.1 g protein MJ⁻¹) had a specific growth rate (SGR) of 1.8 % d⁻¹ (see Chapter 3) compared with 1.6 % d⁻¹ for post-larvae fed with 43.5 % (18.3 g protein MJ⁻¹) and 44.2 % (20.3 g protein MJ⁻¹) dietary protein (see Chapter 4). Compared to protein requirements for sea bream fry (55 %; Vergara et al., 1996a), sea bream growout (45 to 46 %; Vergara et al., 1996b; Jobling and Peruzzi, 2009), and European sea bass growout (43-50 %; Peres and Oliva Teles, 1999; Kaushik, 2002) the protein requirement of striped trumpeter post-larvae was found in this study to be slightly lower (48.4 % from Chapter 3 and 43.5 % from Chapter 4). However, more research is needed to determine the optimum protein requirement of striped trumpeter post-larvae.

The productive protein value (PPV) provides an important assessment of the quality of the ingredients and the formulation of the diet and the bioavailability of the nutrients used. The PPV values of striped trumpeter post-larvae achieved in this study reflect the early stage of diet development and nutrition research on this species. In comparison with other marine aquaculture species of interest the progress achieved in this study is comparable (Table 7.1). Other species being developed as alternatives for the Mediterranean aquaculture industry are the sharpsnout bream (*Diplodus puntazzo*) (Abellan et al., 1994), the white sea bream (*Diplodus sargus*) (Abellan and Basurco, 1999) and the common dentex (*Dentex dentex*) (Abellan, 2000).

Table 7.1: A summary of growth trials conducted on four marine finfish species that are of interest for aquaculture.

Common name	Size of fish (g)	Temperature (°C)	Carcass Crude Protein (% of wet weight)	Specific growth rate (% d ⁻¹)	Productive protein value (%)	Protein to energy ratio (g protein MJ ⁻¹)	Study
Sharpsnout bream ^a	47.0; 277.0	26.0	18.0	1.3	41.4	22.8	Hernandez et al. (2001)
White sea bream ^b	1.0 ; 40.0	22.0	16.8	1.9	24.0	22.3	Sa et al., (2006)
Common dentex ^c	10.0; 95.0	24.0-27.0	17.1	2.2	27.4	23.3	Skalli et al. (2004)
Striped trumpeter ^d	10-50	12.0-18.0	17.7	1.8	29.0	21.8	This study

^a *Diplodus puntazzo*

^b *Diplodus sargus*

^c *Dentex dentex*

^d *Latris lineata*

The protein requirement of fish is more specifically a requirement for the ten essential amino acids for growth, namely, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Wilson, 2002). The ideal protein therefore supplies all of the essential amino acids in the proper proportions. Fish meal is considered a high quality protein source able to supply the indispensable amino acids at the right concentrations (NRC, 1993; Carter and Houlihan, 2001). Amino acid supplementation can increase protein retention when sub-optimal proteins are used or to enhance protein sparing (Hauler and Carter, 2001; Yamamoto et al., 2005). Amino acid composition of tissues provides a useful starting point for estimating amino acid requirements but quantification of requirements for individual amino acids cannot rely on retention figures derived from composition data because this underestimates the requirement for maintenance where amino acids are catabolised for energy (Hauler and Carter, 2001; Conceicao, et al., 2003).

Increasing the inclusion of non-protein energy sources in the diet is used as a strategy to conserve or “spare” protein content for growth (Cho and Bureau, 2001; de la Higuera, 2001). However, there are several caveats to note. A diet with excessive energy limits feed intake resulting in inadequate protein intake which results in protein loss from tissues (Wilson, 2002; Carter et al., 2008). Dietary protein inclusion beyond optimum levels results in excess protein being catabolised for energy and increased excretion rates (Carter and Houlihan, 2001). Factorial models for striped trumpeter post-larvae (see Chapter 5) predict an optimum protein to energy ratio of 21.8 g MJ⁻¹. Results from Chapter 4 did not show a difference in growth, feed conversion and metamorphosis of striped trumpeter post-larvae using dietary lipid inclusion rates of 19 % and 24 %, equivalent to protein to energy ratios

of 18.3 and 20.3 g MJ⁻¹, which were both below the predicted optimum (maximum SGR 1.6 % d⁻¹). The diet used in Chapter 3 had a protein to energy ratio of 22.1 g MJ⁻¹ which resulted in better growth (maximum SGR 1.8 % d⁻¹). The practical relevance of this value is that protein accretion in fish is optimised within a narrow band of protein to energy content and at levels beyond the optimum protein accretion does not increase rather excess protein is degraded and catabolised (Carter and Houlihan, 2001; Carter et al., 2008). In European seabass, Boujard et al. (2004) showed that PPV was enhanced, from 23.2% to 31.1% by decreasing the protein to energy ratio from 26.0 to 20.8 g MJ⁻¹. An earlier study by Dias et al. (1998) on European seabass recommended a protein to energy ratio of 19 g MJ⁻¹. In sea bream, protein retention efficiency was optimised at a protein to energy ratio of 26.0 g MJ⁻¹ (Santinha et al., 1996). These figures indicate that striped trumpeter is similar to European sea bass in terms of protein sparing capability and protein retention.

Other known factors that can influence nutrient retention are feeding regimes, feed properties, the amino acid profile of the protein source and inter-specific differences. Growth trials in my study used a feeding regime of one meal per hour throughout the light period. This was deemed to be optimal in the absence of knowledge of daily feeding rhythms of striped trumpeter. Bolliet et al. (2001) propose that feeding time does affect the growth of fish due to the changing appetite of the fish throughout the day and also due to daily fluctuations in protein synthesis rates. Physical properties of the feed also influence feed intake, these include whether pellets float or sink, texture of the diet and organoleptic qualities (Jobling et al., 2001b). Extruded diets appear superior to pelleted diets resulting in higher lipid content in sea bream (Aksnes et al., 1997) and better growth when a low quality fish meal was used (Vergara et al., 1999)

7.5 Future directions for research

This study has made a first contribution towards understanding the nutrient requirements of striped trumpeter post-larvae and provides empirical data on previously unknown aspects of striped trumpeter nutrition. Future refinements to this study will include digestibility measurements which are an important component of nutrition research to quantify the bioavailability of nutrients (Bureau et al., 2002). If digestibility coefficients are collected in the future they could be applied to the data collected to facilitate comparisons with other species and for refinement of the models developed in my study. Development of systems and techniques for measuring feed intake will also provide data for more accurate models and utilisation predictions. The continued improvement of hatchery protocols has also enabled the research team to produce post-larvae with no jaw malformations which will eliminate the possible confounding influence of jaw malformations from this study.

Diet formulation for striped trumpeter post-larvae is a logical progression from my study with the aim of increasing protein retention and its effects on hastening the metamorphosis process by empirical testing of the optimum digestible protein to digestible energy ratio. Ensuring the quality of the ingredients and the supply of the indispensable amino acids in the correct proportions should improve protein retention as well (Carter et al., 2001). Future diet development studies on striped trumpeter post-larvae will encompass identification of requirements for fatty acids, minerals (e.g. phosphorus) and the effects of diets on excretion rates. In addition to growth, diet formulations should promote vigour in striped trumpeter because of the delayed development of the immune response of post-larvae compared to other marine finfish species (Covello et al., 2009).

Studies of amino acid composition of individual species show contradictory findings, therefore it is prudent to test nutrient requirements and retention for new species (e.g. striped trumpeter, European sea bass and sea bream). For example, Kaushik (1998) found that the amino acid composition of European sea bass, sea bream and turbot (*Psetta maxima*) did not differ. However, Limin et al. (2006) found differences in the amino acid composition of large yellow croaker (*Pseudosciaena crocea*), common sea perch (*Lateolabrax japonicas*), red sea bream (*Pagrosomus major*), Dumeril's amberjack (*Seriola dumerili*), and black grunt (*Hapalogenys nitens*). Further as noted previously, the maintenance requirements are an important component of overall requirements and these may also differ between species.

Amino acid research can facilitate the replacement of fish meal with plant proteins and purified amino acids for use in striped trumpeter diets in light of successful replacement of 95 % of fish meal with plant proteins in European sea bass (Kaushik et al., 2004) and replacement of 60 % of fish meal with plant proteins in sea bream (Dias et al., 2009). The use of plant oils as replacement for fish oil in striped trumpeter diets can also be investigated (Miller et al., 2007, 2008). Rearing striped trumpeter sustainably will further distinguish it as a premium seafood species. Ultimately, the flesh quality of the striped trumpeter should be preserved because of its high concentrations of beneficial *n*-3 fatty acids (Nichols, et al., 2005). The quality of diet formulations should ideally be assessed by the resulting visual and organoleptic qualities of the product (Grigorakis, 2007; Verbeke et al., 2007).

The impacts of climate change on nutrient requirements and utilisation efficiency should also be investigated in light of the the significant influence temperature regime has on physiology of fishes (Jobling, 1994). Climate change is happening and studies have been completed on the impact of warmer oceanic

temperatures on aquaculture in the Tasmanian sea cage industry (Battaglione et al., 2008; Carter et al., 2005; 2008).

7.6 Conclusion

This is the first study on the nutrient requirements and growth of striped trumpeter post-larvae in captivity. The striped trumpeter is an unusual species and no previous studies have been conducted on this life-history stage. Findings from this study reinforce the potential of striped trumpeter as a new marine finfish species for aquaculture. Other favourable attributes of striped trumpeter in culture are its uniform growth and the absence of damage (fin erosion and lesions) caused by aggression and feeding hierarchies. It is envisioned that commercial striped trumpeter aquaculture will come to fruition given the appropriate support from industry, government and research institutions.

This study provided a fundamental knowledge base for future studies of striped trumpeter nutrition and growth data in support of the development of a striped trumpeter aquaculture industry. There are now empirical data on the delicate post-larva life history stage of the striped trumpeter and the techniques for nutrition research will be further refined with experience. This study has achieved its aims and has contributed to the advancement of striped trumpeter and nutrition research.

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